

EFFICACY AND SAFETY OF SPECIFIC IMMUNOTHERAPY TO MOSQUITO BITES

R. Ariano (1), R.-C. Panzani (2)

Summary

■ **Background:** Adverse effects of mosquito bites are often very unpleasant and need a treatment.

■ **Objective:** To evaluate the efficacy of specific immunotherapy (S.I.T) with an extract of the whole body of the mosquito *Aedes communis*.

■ **Method:** Twenty patients having strong local immediate and delayed reactions with in many cases also an allergic rhinitis, were selected. A similar control group was included. In all these patients skin tests, RASTs and nasal provocation tests were carried out with an extract of *Aedes communis* and a battery of the commonest allergens in our area including two insects: *Blatella germanica* (german cockroach) and *Gasterophilus intestinalis* (horse fly).

The specific immunotherapy was carried out by the conventional subcutaneous route.

■ **Results:** After 18 months of S.I.T all the patients reported the disappearance of local reactions and symptoms of allergic rhinitis, which was correlated with a statistically improvement of symptom and drug consumption scores and a decrease of allergenic reactivity by the nasal provocation test.

■ **Conclusions:** S.I.T with an extract of *Aedes communis* produced after 18 months of treatment a significant improvement of allergic symptoms, a decrease of symptom and drug consumption scores and of allergen specific nasal reactivity. S.I.T in mosquito bite allergy appears to be effective and safe in the treatment of both cutaneous and respiratory symptoms.

Key-words: *Aedes communis* - Mosquito bite allergy - Specific immunotherapy.

INTRODUCTION

Respiratory allergy to arthropods is a known but underestimated phenomenon despite the pioneering works of Parlato, (1) Perlman (2), Benaiim Pinto (3) and ourselves (4,5,6,7). On the other hand, allergy to stinging insects have been extensively studied as far as Hymenoptera are concerned with, as a correlate, a precise protocol of specific immunotherapy (S.I.T). Allergy to other stinging insects, such as flea, black fly, ants and mosquitoes is also known but to a lesser extend. Tough sensitization to mosquito bites is more frequent than allergy to Hymenoptera, S.I.T has not been utilized very often. Mosquitoes Culicidae have been the most investigated species, specially in Northern countries of Europe (Finland) with T. Palosuo and T. Reunala, (8-11) and of America and Canada with Peng and Simmons (12). It is also known that mosquitoes can act as aero allergens as demonstrated in India by Agarwal (13).

Mosquitoes are insects which belong to the Diptera order and to the Culicidae family among which we

have three important species: Anopheles, Aedes, Culex. The culicidae family contains more than 2000 species. In Europe, the commonest species are Aedes and Culex but it is possible, in some areas, to find Anopheles, Culiseta and Mansonia (table 1).

Culex species generally reproduce in pits, containers, drums of gardens and aquatic environments with organic materials (septic tanks, drainages). Mosquitoes reproduce particularly during the summer season. When they become adult in winter they take refuge in protected places: humid drains, wine cellars, heated rooms. In our area, the most diffuse and annoying species in houses is *Culex pipiens*; it is active all night long. A second prevalent species in Italy is *Aedes albopictus*, common in Japan, imported a few years ago from the U.S.A and predominant in the countryside.

The first evidences that the bite reactions are immunological in nature were reported by Mellanby (14) in 1946 and McKiel (15) in 1959. Valentine (16) has firmly established the role of saliva venom showing the sensitization to mosquitoes. When their saliva ducts were cut, they did not elicit cutaneous reactions in sensitized subjects. Later, the allergens of mosquitoes were characterized and specific IgE and IgG antibo-

(1) U.O. di Medicina Generale - Modulo di Allergologia ed Immunologia Clinica - A.S.L. n.1 Imperiese - Ospedale "S.Charles" Bordighera (Italy).

(2) Centre de Recherche en Allergologie - 22, rue Edouard Delanglade - 13006 Marseille (France).

dies mostly from the IgG4 subclass were measured by RAST and immunoblotting (17, 18). The main *A. communis* saliva antigens were 22, 30 and 36 kDa which is the most important.

REACTIONS

Bites induce a variety of immunologic skin reactions. The reactions following a mosquito bite are broadly of two types: an IgE mediated wheal and flare appearing after 15 minutes and a more or less lasting papular infiltration after in general 24 hours. However "large local reactions" are the commonest. It is an erythematous itching local swelling of 3 to 12 cms occurring within 12 hours after the bite and persisting one or several days; it seems to be a type I late phase reaction. It is the reaction we generally found in our patients. This type of reaction occurs frequently when adults and mostly children are skin tested with a mosquito extract. Sometimes as reported by Frazier (19) there are less easily definable symptoms such as joint swelling, nausea, headache, dizziness and lethargy. Other symptoms have been described: Skeeter syndrome is a large local inflammation accompanied with fever, and even a respiratory reaction such as asthma (20). Bullous eruptions (21), Arthus's phenomenon (22) and anaphylaxis (23a, 23b) have also been reported.

PURIFICATION OF THE ALLERGENS AND THEIR CROSS REACTIVITY

It has been identified a rather large number of IgE binding allergens ranging from 20 kDa to 70 kDa using a saliva venom which is obviously technically difficult to obtain. As far as we know only two purified and

characterized allergens have been described: Aed 1: (68 kDa apyrase) and Aed 2 (37 kDa) from *Aedes Aegypti*. Thirty seven polypeptides ranging from 16 to 120 KD have been found in whole body extracts of *Aedes* and *Culex* species (Wu, 24). Fewer bands have been detected in saliva with a 62 kDa compound common to the five mosquito species examined (Shen, 25).

It is known that there are cross reactive and species specific allergens. We do not know exactly what is the degree of cross reactivity between on the one hand *Aedes communis* and on the other hand *Culex pipiens* and *Aedes Albopictus*. Peng (26) found studying 10 mosquito species (*Aedes* (*Ae.*) *aegypti*, *Ae. vexans*, *Ae. albopictus*, *Ae. togoi*, *Ae. triseriatus*, *Culex* (*Cx*) *quiquefasciatus*, *Cx pipiens*, *Cx. tarsalis*, *Anopheles* (*An.*) *sinensis*, and *Culiseta* (*Cs*) *inornata*) that the species shared allergens are more important than the specific ones.

NATURAL HISTORY

According to Mellanby, the natural history of cutaneous reactions to mosquito's bites follows the following pattern (table 2). First, the bites cause no reaction (stage 1), next (stage 2) appears a delayed reaction, third both immediate and delayed reactions are induced (stage 3). Then (stage 4) there is only an immediate reaction. Finally at stage 5 no reaction occurs. However since, it is not known what is the average lapse of time, which is probably more or less long according to subjects, between the first mosquito bite and the appearance of tolerance (stage 5) a specific immunotherapy seems to us justified. It appears that there is in general a natural hyposensitization. Natural desensitization was carried out by Mc Kiel *et al.*: repeated voluntary exposure over several months resulted for the majority of the subjects in an improvement.

PHYLUM: ARTHROPODS CLASS: INSECTS

ORDER	HETEROPTERA	SIPHONAPTERA		DIPTERA			
FAMILY	CIMIDAE	REDUVIIDAE	PULICIDAE	CULICIDAE	CERATOPOGONIDAE	SIMULIIDAE	TABANIDAE
SPECIES	CIMEX	TRIATOMA	PULEX IRRITANS	CULEX	CULICOIDES	SIMULIUM EQUINUM	GASTROPHILUS INTESTINALIS
	LECTULARIUS	PROTACTA	CTENOCEPHALIDES	AEDES			
	(HOUSE BUG)	(KISSING BUG)	(FLEAS)	ANOPHELES			

Table 1: Insect classification.

NB: (1) This is a taxonomy of hematophagous insects
(2) House fly belong to the diptera order

According to Palosuo (27) "about half of adult people who had lived exclusively in Finnish Lapland (subject to excessive exposition) were tolerant. In southern Finland the corresponding figure was about 5%. It may take 20 years or so in temperate zone countries".

Since it was not possible to have an extract of both species and owing to the cross reactivity between the different species, we used in this study an extract of *Aedes Communis*. The extract contained both saliva venom and whole body. It is known that there is in general no cross reactivity between the venom and the proteins contained in the whole body. However, years ago, before the impossibility of having purified venom for hymenoptera stings allergy, many allergists used in this case, the whole body of the identified insects.

Since naturally acquired desensitization to mosquito bites occurs after a long term exposure and since several rather successful attempts of S.I.T. have been carried out for a long time, we tried to cure this way some of our patients who had annoying painful reactions. The "natural" desensitization is related to an increase of specific IgG4 antibody to the specific antigens. It seemed to us that S.I.T would help Nature as we have already shown with *parietaria judaica* pollen S.I.T (28). As a matter of fact we found in the literature only thirteen papers dealing with S.I.T for mosquito's bites allergy; the results obtained were in general favorable (29-41). There is a good review on this topic by Hemmer and Jarish (42).

In spite of these drawbacks we started to treat a few patients; then, after the good results obtained at the beginning, we reached the number of 20 patients who are the object of this study.

The aim of this study was to confirm this data and find out whether the efficacy of immunotherapy could be extended to concomitant respiratory reactions.

MATERIALS AND METHODS

PATIENTS

20 patients were accepted for inclusion in our study, the mean age was 26 years. An homogenous control group, of others 10 patients, was selected. In the active group, we have eleven women and nine men, the control group was composed of an equal number of men and women. An informed written consent was obtained from all the patients in both groups.

All these patients reported episodes of persistent pruriginous lesions often painful several hour after being bitten by a mosquito ; the local reaction was between 4 to 10 cms (average diameter) and lasted 24 to 48 hr. Besides, in spring and in summer, they had persistent rhinitis (19 cases associated in 6 cases with a mild asthma). One of the patients has conjunctivitis. Moreover all of these patients had positive skin prick test and RAST to mosquito (*Aedes communis*, Stallergenes) and also positive Nasal Provocation Test to this allergen (table 3).

Other sensitivities (mites, weeds and trees pollens and animal danders) were not exclusion criteria. . None of them reported clinical reactions to the stings of horse fly (Tabanidae), black fly (Simuliidae), flea, Hymenoptera or other unidentified insects.

Subjects with a disease that would interfere with the evaluation of clinical symptoms, such as rhinitis medicamentosa, sinusitis or large obstructive nasal polyps, pregnancy or breast feeding, and serious systemic disease, were discarded.

ALLERGENIC EXTRACTS

All of the extracts were supplied by Stallergenes Laboratories (Paris, France). The allergen preparation

The five – stage sequence of skin reactivity in hosts exposed to repeated mosquito feeding*

	IMMEDIATE REACTION (15 MIN)	DELAYED REACTION
STAGE I (NON SENSITIZED)	-	-
STAGE II	-	+
STAGE III	+	+
STAGE IV	+	-
STAGE V (TOLERANCE)	-	-

Table 2: Natural immunological development of mosquito's bites.

* After Mellanby and Mc Kiel.

ACTIVE GROUP

N°	GENDER	PRICK TEST	AGE	RAST CLASS	SYMPTOMS	OTHER ALLERGENS
1	F	4	47	4	AR	DPF OE BLA GI
2	F	3	27	4	AR	DPF CU
3	M	3	17	4	AR	DPF CU
4	F	4	19	4	AR	PA GR BE BLA GI
5	F	4	16	4	AR	DPF PA OE BLA GI
6	M	4	26	3	AR	DPF BLA GI
7	M	4	31	3	A	PA GR CU
8	F	3	27	3	C	DPF BLA GI
9	M	3	20	3	A	GR DPF GLA GI
10	F	4	20	3	A	No
11	F	3	21	3	AR	DPF GI
12	F	4	21	3	AR	No
13	M	4	16	3	AR	GI
14	F	3	20	3	A	GI
15	M	3	28	3	AR	DPF BLA GI
16	M	3	38	3	AR	BLA GI
17	M	3	17	3	A	GR CU BLA GI
18	F	3	31	3	A	DPF BLA
19	M	4	37	3	AR	PA BLA GI
20	F	3	36	3	AR	BLA GI

CONTROL GROUP

N°	GENDER	PRICK TEST	AGE	RAST CLASS	SYMPTOMS	OTHER ALLERGENS
1	M	3	25	4	AR	DPF PA BE BLA GI
2	M	4	36	4	AR	GR PA OE GI
3	M	3	16	4	A	DPF BLA GI
4	F	4	15	3	AR	DPF BLA GI
5	F	4	33	3	AR	GI
6	F	3	33	3	A	DPF BLA GI
7	F	4	38	3	AR	PA GR BE BLA GI
8	M	3	17	3	AR	No
9	F	3	17	3	A	GI
10	M	3	20	3	AR	GI

LEGEND

A	ASTHMA	DPF	DERMATOPHAGOIDES PTERON. OR FARINAE
AR	ALLERGIC RHINITIS	CU	CUPRESSACEAE
C	CONJUNCTIVITIS	BE	BETULA ALBA
GR	GRASSES POLLEN	BLA	BLATELLA GERMANICA
OE	OLEA EUROPEA	GI	GASTEROPHILUS INTESTINALIS (HORSE FLY)
PA	PARIETARIA JUDAICA		

Table 3: Clinical data of our patients.

was based on Good Manufacturing Practice (GMP) with an in-house reference standard. An allergenic extract is defined as 100 I.R. ml when, used by prick-test using a STALLERPOINT on 30 subjects who are sensitive to the allergen, it gives a weal size of 7 mm (geometric mean). The skin reactivity of the subjects is simultaneously shown with 9% codeine phosphate or histamine chloride at 10 mg/taken as a positive control.

However, we did not know what was the percentage of saliva venom in the whole body extracts used in this study.

It is obvious that, for the collecting of saliva venom is currently too tedious and impractical, a new method of standardizing the extracts from whole body should be found. As suggested by Peng *et al.* (43) mosquito's head and thorax contained more saliva proteins.

SKIN TESTING

The prick testing was performed according to the method of the subcommittee on Skin Tests of the European Academy of allergy and Clinical Immunology using standardized lancets (Dome Hollister Stier, west Haven, CT, USA).

We adopted the following scores: a 3+ reaction corresponded to the weal and erythema of histamine (1 mg for the 1-ml prick test). A 4+ reaction corresponded to a reaction greater than that caused by histamine. A 2+ reaction was 75% and a 1+ reaction was half the size of the histamine reaction.

Histamine hydrochloride (1 mg/ml) and buffer solution were tested as controls of positive and negative skin reaction respectively. The distance between test sites of Histamine and *Aedes Communis* was more than 5 cm. Skin prick test results were recorded after 15 to 20 minutes. The mean weal diameter (MD) was calculated according to the formula $(D + d)/2$ where D represents the largest (longitudinal) diameter and its midpoint orthogonal diameter.

Skin prick tests (SPTs) were performed with the commonest inhalant allergens (*Dermatophagoides pteronyssinus*, *Parietaria judaica*, *Cynodon dactylon*, *Lolium perenne*, *Olea europea*, *Artemisia vulgaris*, *Ambrosia elatior*, *Cupressus sempervirens*, *Alternaria alternata*, Cat, Dog). The following insects besides *Aedes communis* were included in this panel: *Aedes communis*, *Blattella germanica* (german cockroach), *Gasterophilus intestinalis* (horse fly). Table III shows the clinical data corresponding to the 20 patients of the treated group and the 10 patients of the control group. All the patients had a positive skin test and RAST to asthma. With the same extract, we had in all cases, a positive nasal provocation test.

IN VITRO TESTS

Specific serum IgE determinations were performed by enzyme immune analysis CAP FEIA (Pharmacia - Upjohn Uppsala Sweden) using an extract of *Aedes communis* and following the manufacturer's instructions. Specific IgE equal to or higher than 0.35 KU / l was considered positive.

NASAL PROVOCATION TEST

A specific nasal provocation test was carried out on all patients, before and after treatment, with an extract of *Aedes Communis*. The extracts were used at several concentrations: 1, 10, 100/ml (with non phenolated saline diluent and spray of 100 microl/constant volume). The patients had to be symptom-free. Tests were performed with instillation with a pressurized spray into one nostril of increasing concentration at 15 minute intervals. A control test with diluted buffer was performed into the opposite site. The threshold dose of allergen able to elicit two out of four of the following nasal symptoms (sneezing, obstruction, itching, rhinorrhea) was determined after two peak inspiratory flow meter measurements. We used the Youlten peak inspiratory flow meter (PIFRn meter). It offers some advantages over the expiratory flow rate meter especially when used in provocation test and it is easier to handle compared with standard rhinomanometry. The results are expressed as the mean values of three consecutive registrations. A test was considered positive when we observed a drop in the basic value of 20% and/or elicit at least two symptoms among the four followings: sneezing, obstruction, itching, rhinorrhea.

BITE CHALLENGE PROTOCOL

This technique which is rather cumbersome was not carried out in our study for the obvious reason that the field bites were negative in all the treated cases.

■ **Specific immunotherapy (SIT):** Specific immunotherapy with a mosquito whole body extract (*Aedes communis*) was initially carried out with weekly subcutaneous injections, beginning at a concentration of 0.01- 1 I.R. and increasing the dose progressively for two months, according to the following schedule (table 4) The maintenance treatment was carried out with weekly injections at a concentration of 10 IC for fourteen month. The total length of treatment was 18 months and the cumulative dosage received by the patients was therefore 120 I.R.

INITIAL	PHASE (weekly)
0,01 IR/ml	0,10 0,20 0,40 0,80
0,1 IR/ml	0,10 0,20 0,40 0,80
1 IR/ml	0,10 0,20 0,40 0,80
10 IR/ml	0,10 0,20 0,40 0,80
Maintenance concentrate (monthly)	
10 IR/ml	0,80

Table 4: Schedule of treatment.

■ **Symptom and drug scores:** Symptom and drug scores were recorded daily on a cart. Rhinitis i.e., sneezing, pruritus, nasal discharge, nasal obstruction was scored 0-3. Eye redness, itching, tears and swelling were also scored 0 to 3, as well as cough, wheezing, sputum, breathlessness. A score 0 indicated absence of symptoms. Drugs taken were scored with 1 point for every administration.

■ **Statistical analysis:** Statistical evaluation was done by Mann-Whitney U-test (two tailed probabilities) for the intergroup comparisons and the Wilcoxon signed rank for intragroup comparisons at the different time of observation. The chi-square test was used to test the significance of differences among the overall evaluation stated at the end of the trial. The level of significance chosen was $p < 0.05$.

RESULTS

After 18 months of immunotherapy all the 20 patients, who received the specific immunotherapy, reported the disappearance of persistent cutaneous lesions and respiratory or ocular symptoms after been bitten by a mosquito. On the contrary none of the 10 patients in the control group, who have not received the treat-

gitis. We did not carry out a controlled mosquito bite challenge before and after the treatment but no patient in the active group reported to have experienced any reaction after being stung.

Moreover, at the end of the treatment, we observed a statistically significant improvement of symptom and drug consumption scores, only in the active group and not in the control group. The data are shown in the table 5. with the results of statistical analysis with Wilcoxon test. Also the intergroup comparisons, with Mann-Whitney U-test, showed a significant difference between the two groups, before the treatment.

Moreover, a significant increase ($p < 0.05$) in the threshold dose to specific nasal challenge in comparison with baseline values was observed in actively treated group but not in the control group, after the period of treatment (table 6).

All patients completed the period of 18 months of immunotherapy and no local or systemic side-effects were reported in the active group.

		Before	After	Statistical analysis
ACTIVE GROUP	Symptoms	18.14 (sd 2.61)	6.43 (sd 3.51)	$P < 0.05$
	Drugs	5.29 (sd 2.15)	2.43 (2.15)	$P < 0.05$
CONTROL GROUP	Symptoms	18.80 (sd 2.68)	18.80 (sd 2.68)	NS
	Drugs	5.60 (sd 1.14)	5.40 (sd 0.89)	NS

Table 5: Mean monthly symptom and drug scores in active and control before and after the treatment.

* Wilcoxon test

NS: Not significant

sd: standard deviation

	ACTIVE GROUP		CONTROL GROUP	
	BEFORE	AFTER	BEFORE	AFTER
MEAN 7.86 DOSES (sd 2.67)	32.86 (sd 7.56)	*	8.0 (sd 2.74)	9.0 (sd 2.24)
Difference	25.0	**	1.0	

Table 6: Changes in nasal provocation tests in both groups before and after the treatment.

* $p < 0.01$ Wilcoxon test; ** Mann-Whitney U-test; ns = not significant
sd = standard deviation.

DISCUSSION

S.I.T with whole body extract has already been practiced. Most of the

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