

Immunology and treatment of mosquito bites

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Summary

Cutaneous reactions to mosquito bites are usually pruritic weals and delayed papules. Arthus-type local and systemic symptoms can also occur but anaphylactic reactions are very rare. Both clinical and experimental evidence suggest that the various bite reactions result from sensitization to the mosquito saliva injected into the skin during feeding. Recent immunoblot studies have shown both IgG- and IgE-class anti-mosquito antibodies, but their species-specificity and clinical importance is at present unknown. In addition to an Arthus-type mechanism, both cutaneous late-phase reactivity and cell-mediated immunity may be involved in the pathophysiology of delayed mosquito-bite lesions. Cutaneous sensitization to mosquito bites can be divided into five different stages ranging from the stages of immediate wealing and delayed bite papules, to the stage of non-reactivity. No desensitization treatment is generally available for mosquito allergy but it has recently been shown that cetirizine, a potent non-sedating antihistamine, is effective against the wealing and pruritus caused by mosquito bites.

Introduction

Female mosquitoes need blood for optimal development of their eggs and, in tropical climates are well known vectors of many important diseases such as malaria and yellow fever. In the northern areas of Europe, Asia and North America, mosquitoes are very numerous and they can also be found in abundance in coastal salt-marshes along the Atlantic coast and the Gulf of Mexico [1]. In these areas mosquito-borne disease transmission is not of major importance, but mosquito attacks often create difficulties for local people and especially for tourists. The bites cause cutaneous reactions which vary from mild weals and papules to severe Arthus-type reactions with systemic symptoms [1,2]. The bite lesions are usually accompanied by intense pruritus, thus even small papules may cause much discomfort. In Scandinavian countries, the majority of people react to mosquito bites and in every mosquito season many people have to seek medical advice for the treatment of bite lesions.

The mosquito-bite reactions are generally considered to be immunological in nature [1–3]. When feeding, mosquitoes inject saliva into the skin and this seems to sensitize both animals and man [3–6]. Under experi-

mental conditions, the cutaneous response to the bites ranges from immediate weals to delayed papules or no reaction at all [7,8]. Anecdotal evidence suggests that a similar sequence of sensitization occurs in nature after exposure to mosquitoes. The antigens in mosquito saliva and the mechanisms causing the various bite reactions have remained obscure. The weal reaction is commonly thought to be histamine-mediated but there are only a few reports on the occurrence of IgE-class or other anti-mosquito antibodies (AMAB) in man. Similarly, the cellular events of delayed mosquito-bites have not been well characterized.

Clinical types of mosquito-bite reactions

Normal bite reactions

Several studies performed over the last 40 years show that a characteristic sequence of events takes place in all subjects exposed to mosquito bites over a period of time. In England, Mellanby [7] exposed 25 volunteers to *Aedes aegypti* mosquitoes, a species which is found outside the U.K. only. The initial bites caused no reaction, but with subsequent bites a delayed cutaneous lesion appeared. After repeated bites for about 1 month an immediate weal developed, and then, with further exposure for several

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Table 1. Different stages in the development of immediate and delayed cutaneous reactions to mosquito bites

Immunological stage	Immediate reaction	Delayed reaction
Stage 1	—	—
Stage 2	—	+
Stage 3	+	+
Stage 4	+	—
Stage 5	—	—

After Mellanby [7] and McKiel & West [8].

months, the delayed reaction disappeared. Mellanby [7] classified the reaction patterns into four distinct stages, but later McKiel *et al.* [8] extended this classification of man's reaction to mosquito bites to five different stages (Table 1). Stage 5 was not reached during the 1 yr of Mellanby's [7] experiments, but it would seem that the non-reactivity of the native inhabitants of mosquito-infested areas such as Lapland represents this ultimate stage [1,3,9].

Abnormal bite reactions

The usual immediate reaction is a weal and flare, the diameter of which varies from 2 to 10 mm (Fig. 1). Some people may have large local swellings on the bite sites but immediate systemic symptoms seem to be very rare in direct contrast to bee stings [10], for example. There are a few case reports of anaphylactic-type reactions from mosquito bites [1]. In our series, collected over 5 yr, there was only one patient, a child aged 4 yr who presented with generalized urticaria, an attack of asthma and low blood pressure after 1–3 mosquito bites.

Delayed, small but pruritic papules are very common after mosquito bites (Fig. 2). These appear several hours after the bites and usually last 1–3 days, but in a few subjects they may persist for several days, sometimes even weeks. After repeated bites, the old bite sites may show flare-ups and the clinical picture is that of a lichen urticatus or strophulus [1,2]. The cutaneous manifestations of Arthus-type reactions can be small or large vesicular, pustular, haemorrhagic or necrotic lesions with intense surrounding erythema (Fig. 3). Joint swelling, lymphadenopathy and fever may accompany these lesions [1,11,12]. An erythema multiforma-type clinical picture and anaphylactoid purpura have also been reported [1].

Blisters or tense bullae can occur, especially on the lower legs, and in England the condition known as



Fig. 1. A close-up view of a weal caused by a bite of *Aedes communis* mosquito.



Fig. 2. Three-day-old papular mosquito bite lesions on the ankle of a child.

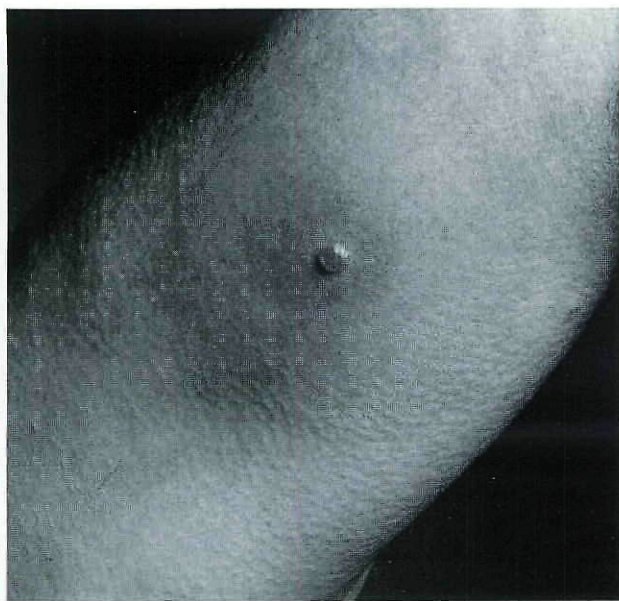


Fig. 3. Blistering, erythematous lesion 1 day after the bite of *Culex pipiens* mosquito.

seasonal bullous eruption was recently shown to be caused by *Aedes detritus* mosquitoes [13].

Exaggerated reactions to mosquito bites are known to develop in certain disease conditions and are accompanied by an altered immune function. Patients with chronic lymphocytic leukaemia belong to the age group which usually tolerates mosquito bites well. However, they may exhibit severe, delayed bite reactions which can appear before the malignancy has been diagnosed [14]. Recently, Japanese investigators focused attention on severe, Arthus-type mosquito hypersensitivity appearing in childhood and culminating, in many cases, in malignant histiocytosis [12,15]. Interestingly, altered cutaneous reactions to mosquito bites have also been reported in three patients with AIDS, after receiving zidovudine treatment [16].

Immunology of mosquito-bite reactions

Antigens

Hudson *et al.* [5] confirmed that mosquito saliva is a source of antigens which produce typical bite reactions in man. They cut the salivary ducts, after which no cutaneous reactions were observed in sensitized subjects. Thereafter, several attempts to characterize the salivary antigens were made. Newsome *et al.* [17] isolated a glycoprotein with a minimum molecular weight of 33.5 kD from the saliva of *A. aegypti* mosquitoes and showed

that this elicited skin reactions in sensitized subjects. Racioppi and Spielman [18] demonstrated 12 major polypeptides in the saliva of the same species and showed that they were all immunogenic when injected into guinea-pigs. Recent studies with sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting have yielded more information about the mosquito antigens. In SDS-PAGE, whole-body extracts (WBE) of *Aedes* and *Culex* mosquitoes show at least 26–37 polypeptides with molecular weights ranging from 16 to 120 kD [19].

Similarly, Shen *et al.* [20] also found multiple bands when using WBE from several mosquito species, but only a few bands were detected in salivary gland extracts. Penneys *et al.* [21] used salivary gland extracts from five different mosquito species and serum samples from human subjects as a source of AMAB. They could identify several antigenic components ranging from 14 to 126 kD but only a 62 kD compound was common to all five mosquito species examined. We immunized rabbits with the bites of *Aedes communis* mosquitoes and could detect a 21.5 kD antigen in the WBE of these mosquitoes with the immunoblot technique [22].

A. communis mosquitoes cause most bite reactions in Finland and we recently showed that this 21.5 kD antigen was also responsible for IgG-class AMAB response in man [23]. Our preliminary results showed that the saliva of *A. communis* mosquitoes contains three major antigenic components, one of which is the 21.5 kD protein [H. Brummer-Korvenkontio, unpublished observation]. At present, the species-specificity of the antigens detected by us remains open and nothing is known about the function of these proteins in mosquito saliva. The possibility exists that they may have anticoagulant or esterase activities [24].

Anti-mosquito antibodies

Precipitating antibodies have been demonstrated after experimental exposure to *A. aegypti* mosquito bites in guinea-pigs and rabbits, and their appearance seems to correlate with the development of skin reactions [25]. Ailus *et al.* [9] showed the occurrence of precipitating antibodies to *A. communis* mosquitoes in man and presented evidence that their occurrence was related to the degree of mosquito exposure. People living in Lapland had a lower frequency of precipitating AMAB, suggesting that massive exposure may result in suppression of humoral antibody response. Penneys *et al.* [26] used an immunohistochemical technique to demonstrate circulating IgG-class anti-salivary gland AMAB and they also found little antibody binding in subjects with a history of extensive mosquito exposure.

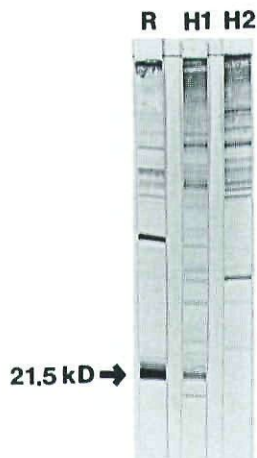


Fig. 4. Identification of IgG-class anti-mosquito antibodies to 21.5 kD antigen of *Aedes communis* mosquitoes by immunoblotting. The antibody is detected in the serum from a rabbit immunized with *A. communis* bites (lane R) and in the first (H1) but not in the second (H2) serum obtained from human adults exposed to mosquitoes in nature.

We recently used an immunoblot technique to measure AMAB response in man. Infants never exposed to mosquitoes had no antibodies to the 21.5 kD salivary antigen of *A. communis* mosquitoes, but in older children and adults the frequency was about 50% (Fig. 4). These antibodies were mainly of the IgG4 subclass but their clinical significance is unknown at present [23].

The immediate mosquito bite reaction is a weal, but the evidence that it is mediated by IgE antibodies and histamine release is far from conclusive. Successful passive transfer of reactivity has been carried out with the Prausnitz-Kustner test under mosquito feeding or using extracts as the challenge [2,11,27].

Suzuki *et al.* [27] showed, using RAST, IgE antibodies to *Aedes*, *Anopheles* and *Culex* mosquitoes in one highly mosquito-hypersensitive child. However, attempts to detect IgE AMAB by RAST or an immunohistochemical technique have usually failed [23,26]. We succeeded in demonstrating low levels of IgE AMAB (0.5 PRU/ml) by RAST (Pharmacia Diagnostics, Uppsala, Sweden) in only one patient with a severe type I allergy to mosquito bites. In contrast to the weakly positive RAST, this patient had a clearly positive basophil histamine release test (80% histamine release) and also showed IgE antibodies to a 21.5 kD salivary antigen in immunoblotting when the same WBE of *A. communis* mosquitoes was used as an antigen [28]. Recently, Wu and Lan [19] also demonstrated IgE antibodies in two mosquito-bite sensitive patients using the WBE of *Aedes albopictus* mosquitoes. Immunoblotting with subsequent autoradio-

graphy showed the IgE antibodies bound to 52 and 56 kD mosquito proteins. Shen *et al.* [20] used the salivary gland extract of the same species and detected IgE antibodies in only one of the 15 sera examined. These divergent immunoblot results show that there is an apparent need to use pure salivary antigens to discover the IgE antibody response caused by mosquito bites and that perhaps resulting from the inhalation of crossreacting insect material [29]. If the frequency of IgE AMAB appears to be low in patients experiencing abnormal immediate reactions to mosquito bites, this is in sharp contrast to the allergy caused by hymenoptera stings, or inhaled insect allergens such as chironomid midges [10,29,30].

Cellular immunity

Several findings suggest that cellular immune mechanisms play a similar role in delayed reactions to mosquito bites as they do in hymenoptera allergy [31]. In guinea-pigs, delayed mosquito reactivity has been successfully transferred with spleen cells to recipient animals [32]. In man, most of the subjects exhibiting delayed bite reactions also seem to have a delayed response when skin is tested with WBE of the same species [2]. Moreover, the delayed papular bite reactions show dense perivascular infiltrates of lymphocytes and histiocytes which, in more intense reactions, may extend to subcutaneous fat and also be accompanied by varying amounts of eosinophils [2]. No detailed immunohistochemical studies have been performed to characterize the cellular infiltrates but, in one exceptional mosquito-hypersensitive case, the immunophenotyping showed that most of the infiltrating lymphocytes had natural killer cell phenotype [12].

In hymenoptera allergy many patients develop large local, and often persistent, reactions at the sting sites. In addition to delayed skin-test reactivity to the venom, many of these patients also show positive lymphocyte proliferation tests [31]. To investigate cell-mediated immunity to mosquitoes we performed lymphocyte proliferation tests with the WBE of *A. communis* mosquitoes. The results were positive in three of the five patients exhibiting delayed response to mosquito bites [23]. These preliminary *in-vitro* results suggest that cellular immune mechanisms can, at least in some subjects, be responsible for mediating delayed cutaneous responses to mosquito bites. It is, however, possible that some of the delayed bite reactions, and especially those occurring in combination with strong immediate reactions and many eosinophils, may represent cutaneous late-phase reactions [33]. To our knowledge, no studies have been performed to elucidate this mechanism in delayed mosquito-bite reactions and, therefore, both the cell-mediated immunity and late-

phase cutaneous reactions are good candidates for future research.

Treatment of mosquito-bite reactions

Palliative antipruritic creams, sticks and aerosol containing hydrocortisone or antihistamines are widely used for the treatment of mosquito-bite skin lesions. There are, however, only a few controlled studies on the effects of these products on insect bites and, for topical antihistamines, the results were mainly negative [34]. Recently, Coulie *et al.* [35] carried out a double-blind, placebo-controlled study with oral cetirizine on experimental mosquito bites in volunteers. They showed that 10 mg b.i.d. cetirizine significantly reduced pruritus but had no clear effect on the intensity or duration of mosquito-bite induced skin lesions. Our recent placebo-controlled study in 23 volunteers exposed to mosquito bites in nature showed that 10 mg cetirizine significantly reduced both pruritus and wealing but, again, no effect was found on the delayed symptoms. The effect of cetirizine on the weal response provides additional evidence that the immediate reaction to mosquito bites is mediated by histamine. In addition to its anti- H_1 activity, cetirizine also markedly inhibits cutaneous late-phase reactions [36]. Future research is needed to confirm if cetirizine is also useful for patients experiencing large cutaneous reactions after mosquito bites. The clear benefit from a potent, non-sedating antihistamine is that the drug can be taken prophylactically when going to areas where mosquito bites cannot be avoided. Repellents containing diethyltoluamide are effective but, especially in children, their potential toxicity is high [37] and even if properly used, repellents cannot provide total protection from mosquito bites.

Acknowledgments

We greatly appreciate the expert technical assistance of Maili Lehto. This study was supported by a grant from the Finnish Foundation for Allergological Research.

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