

15 | Biting Insect Allergens

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INTRODUCTION

Allergic reactions to insect bites are much less common than reactions to insect stings. Several studies suggest that severe bite reactions occur about 50 times less commonly than severe sting reactions. Many of the clinical aspects of biting insect allergy have been thoroughly discussed in a 2003 review (1). In this chapter, the main focus will be on which insects are important, the known allergens and salivary components, and the appropriate use of immunotherapy. There are more than 14,000 species from 400 genera of blood-feeding arthropods. The most important hematophagous insects belong to the orders Diptera (flies), Hemiptera (bugs), and Siphonaptera (fleas). Ticks of the order Acarina of the class Arachnida will also be considered, although they are not insects. Many other bugs of the order Hemiptera and some beetles, especially aquatic species, of the order Coleoptera occasionally bite humans, but allergic reactions have not been reported. In addition, many larval forms may bite, but again allergic reactions to these bites are extremely rare. Allergic reactions to bites have been ascribed to other arachnids, but definitive evidence is lacking to demonstrate IgE antibodies against centipede and millipede bites. There probably are rare cases of IgE-mediated allergy to spider bites, but there are no published systematic studies.

TAXONOMY OF BITING INSECTS

Diptera, Flies

Many flies are hematophagous. In almost all cases only the females bite, requiring a blood meal to develop eggs. The more common biting flies are outlined in Table 1. A blackfly, deerfly, and horsefly are illustrated in Figures 1 to 3.

Hemiptera, Bugs

There are two important families of biting bugs in North America. The first are variously called kissing bugs, assassin bugs, conenose bugs, vinchucas, or reduviid bugs and are members of the family Reduviidae. There are 39 genera of which the most important are *Triatoma* (Fig. 4) and *Reduvius*. The Latin American genera *Rhodnius* and *Panstrongylus* are important members of this family. The second family of blood-sucking bugs is Cimicidae or bedbugs. There are seven genera, and the species *Cimex lectularius* is the most infamous human bedbug.

Siphonaptera, Fleas

The fleas are almost all parasitic insects with 74% of species associated with rodent hosts, and about 6% with avian hosts. The species associated with humans are members of the superfamily Pulicoidea, family Pulicidae. The most common are the dog and cat fleas *Ctenocephalides canis* and *Cte. felis felis*. *Pulex irritans*, a parasite of carnivores, is sometimes called the human flea. Fleas of the genus *Tunga* are found in Central and South America.

Arachnids

Many species of hard and soft ticks and chiggers bite humans. Allergic reactions to these bites are extremely rare, although they have been reported (2,3) from many regions.

Table 1 Biting Flies (Diptera)

Common name	Family	Genera
Mosquito	Culicidae	<i>Aedes, Culex, Anopheles</i> , others
Blackfly	Simuliidae	<i>Simulium, Prosimulium, Cnephia</i>
Biting midge	Ceratopogonidae	<i>Culicoides</i> , others
Horsefly	Tabanidae	<i>Tabanus, Hybomitra</i>
Deerfly, yellow fly	Tabanidae	<i>Chrysops</i>
Sand fly	Psychodidae (Phlebotominae)	<i>Lutzomyia, Phlebotomus</i>
Bottle and warble fly	Oestridae	<i>Dermatobia</i> , others
Stable fly	Muscidae	<i>Stomoxys, Haematobia</i>
Tsetse fly	Glossinidae (Muscidae)	<i>Glossina</i>



Figure 1 (See color insert.) Photograph of a blackfly, *Simulium*; note the humped appearance. Source: Courtesy of Jerry F. Butler, University of Florida.

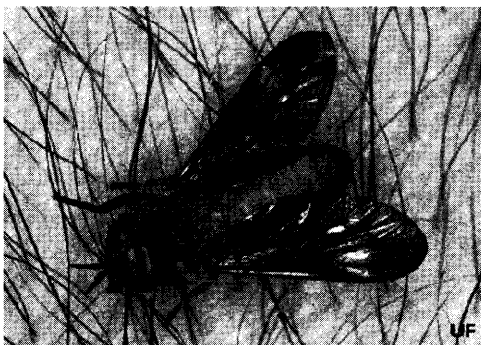


Figure 2 (See color insert.) A deerfly, *Chrysops*, in biting position. The insect is usually yellow or green, and the bite is painful. Source: Courtesy of Jerry F. Butler, University of Florida.

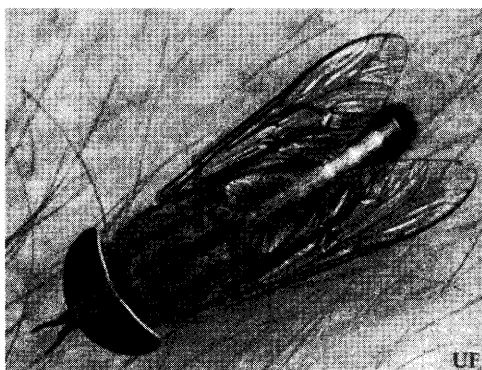


Figure 3 (See color insert.) A horsefly, *Tabanus*, biting. Horseflies are typically larger than deerflies, have very noisy flight, and the bites are quite painful. Source: Courtesy of Jerry F. Butler, University of Florida.

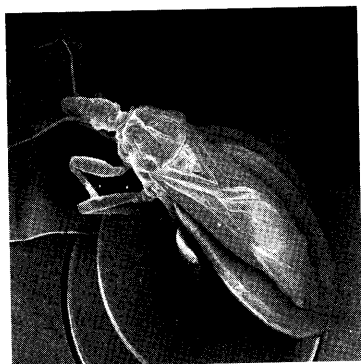


Figure 4 Scanning electron micrograph of a kissing bug, *Triatoma protracta*. Bites are painless, typically occurring while sleeping. The insect's definitive host is the wood rat. *Source:* Courtesy of C. Demetry and R. Biderman, Worcester Polytechnic Institute.

IDENTIFYING BITING INSECTS

The identification of biting insect can be extremely difficult, even with representative specimens. Deerflies, horseflies (Fig. 1), and stable flies all cause immediate pain when they bite. Mosquitoes can usually be recognized, but identification of species may require an expert. Identification of flea species is the realm of specialists. Kissing bugs typically bite painlessly, most commonly while the victim is sleeping. Useful identification guides with many illustrations are available for hobbyists including Borror and White's A Field Guide to the Insects and the National Audubon Society Field Guides. The much more technical and comprehensive reference to insects of North America by Arnett (4) is recommended for those with a serious interest. Keys to various groups are available in the entomology literature and vary widely in quality and usability. Most states have official entomologists, usually with the Department of Agriculture, who are many times willing to assist in insect identification for medical purposes. There are also entomologists at many land-grant universities, who are willing to assist with insect identification.

GEOGRAPHIC DISTRIBUTION OF SOME BITING INSECTS

Mosquitoes are cosmopolitan with species found in almost all land areas of the world. Fleas are found in most areas of the world, except in very dry climate areas. Blackflies are found in the northern United States, in most of Canada, and in much of Northern and Central Europe and Asia; in tropical areas they require the presence of rapidly running water to breed. Horseflies and deerflies are found in most temperate areas of the world. Tsetse flies are only found in tropical Africa and a few laboratories in the United States and Europe.

Ticks are found around wooded areas and are commonly carried by dogs, birds, and deer. Various species are found distributed in different areas of the world. Sand flies and biting midges are also found in many areas, especially around beaches and livestock.

Although bugs of the reduviid group are found in many areas, almost all cases of allergic reactions to bites are found in the southwestern United States, Hawaii, Mexico, and Central America. These insects are dependent on the distribution of their hosts, for example, the wood rat in California for *Triatoma protracta*. Other species feed on dogs, cats, mice, opossums, and armadillos.

SALIVARY COMPONENTS AND ALLERGENS OF BITING INSECTS

According to Ribeiro (5), blood feeding evolved independently multiple times among hematophagous arthropods. A variety of anticlotting factors, platelet aggregation antagonists, and vasodilators developed to counter the host's hemostatic and immunomodulatory factors (6). In addition, arthropod salivas contain digestive enzymes (7) and hyaluronidase. One unsuspected property of some insect salivas is enhancement of infectivity of parasites carried by the arthropod (8). Sand fly saliva decreases the minimum effective dose of *Leishmania major* in mice by several orders of magnitude. Studies suggest that immune reactions to biting insect

saliva may increase resistance to infection with protozoans (9). In 2002, the first complete genome sequence of a biting insect, the malaria mosquito, *Anopheles gambiae*, became available (9). The set of proteins expressed by the salivary glands have been named the sialome (10) by Ribeiro (11) and have been mapped for mosquitoes (11), kissing bugs (12), and ticks (13).

Mosquitoes

Many of the characterized protein components of mosquito saliva are described in Table 2. A number of the proteins appear to be related to either digestive functions such as maltase, amylase, and esterase or inhibition of hemostasis such as tachykinin, factor Xa inhibitor, purine nucleosidase, and apyrase or adenosine triphosphate diphosphohydrolase, which inhibits ADP-dependent platelet aggregation (11,14–19). Protein D7 contains two insect pheromone/odorant-binding protein domains and is expressed in a number of different sizes (11,19). D7 proteins appear to be major allergens in most species.

There are numerous published studies of IgE-binding components of various mosquito extracts. Some are performed with "saliva," some with salivary gland extract, some with thorax extract, and some with whole-body extract. Numerous species and at least four genera have been investigated. Table 3 is a compendium of the major and shared allergens in

Table 2 Selected Protein Components in *Aedes aegypti* Mosquito Saliva Identified by Two-Dimensional Electrophoresis Followed by Trypsin Digestion and Mass Spectrometry, and Also by cDNA Expression

Component	Genbank identifier (gi)
Protein D7 11	16225992
Protein D7 12	118216
Antigen 5-like protein	18568284
Apyrase	1703351
Purine nucleosidase	21654712
Protein disulfide isomerase	94468800
Heat shock protein 70 kDa	94468818
Adenosine triphosphate phosphatase	
Beta unit	94468834
Alpha unit	94468442
Angiopoietin-like protein	1858298
Salivary serpin 1	18568304
Salivary serpin 2	94469320
62-kDa proteins	18568300 and 2
Amylase	2190949
Adenosine deaminase	18568326
C type lectins	94468370, 18568318
34-kDa protein 1	94468642
34-kDa protein 2	18568296
Angiopoietin-like protein 2	94468352
30.5-kDa salivary protein	61742033
30-kDa salivary antigen	18568322

Source: From Ref. 11.

Table 3 Molecular Weights (in kDa) of IgE-Binding Components in Mosquito Extracts from Various Species

<i>Aedes aegypti</i>	<i>Aedes vexans</i>	<i>Aedes communis</i>	<i>Culex tarsalis</i>	<i>Culiseta inornata</i>
Major allergens				
65	65	36	43	65
48	43	30	17	40
34 = D7	38	22	15	34
31				
15				

Minor allergens shared by at least three species 160, 110, 65, 62, 50, 46, 40, 32.5, 24, 17, and 15 kDa. Allergens have not been purified; data mainly from immunoblot experiments, some, e.g., D7, have been cloned or expressed.

Source: From Refs. 21–26.

immunoblot experiments for five species and thirteen species, respectively. It appears that D7 protein is an important allergen in *Aedes*, *Culex*, and related mosquitoes, and that apyrase may also be an allergen. None of the other IgE-binding bands has been definitively characterized. A 2004 study (20) reported on 14 individuals with acute systemic reactions to mosquito bites; all showed IgE antibodies specific to saliva from at least one of the five species tested.

Blackflies

Studies on the saliva of blackflies are very limited. Cupp et al. (27,28) isolated and cloned a major protein of molecular weight 15,351 Da with strong vasodilator activity manifested by rapid and persistent induction of erythema. The enzyme apyrase is also found in blackfly salivary gland secretions. Wirtz (29) demonstrated high contents of histamine, putrescine, spermine, *N*-monoacetylspermine, and spermidine, as well as the presence of proteins with esterase activity. Almost all reactions to blackfly bites are not IgE mediated, and the dermatologic reactions have been classified into six forms by Farkas (30). These are edematous, erythematous-edematous, "erysipeloid," inflammatory-indurative, hemorrhagic (plaques, nodules, or vesicles), and allergic.

Horseflies and Deerflies

Deerfly saliva contains chrysoptin, an inhibitor of ADP-induced platelet aggregation that inhibits fibrinogen binding to the glycoprotein IIb/IIIa receptor on platelets (31,32). The recombinant protein with a molecular mass of 65 kDa, the same as that of the natural protein, has been expressed in insect cells. This may be a protein similar to the 69-kDa IgE-binding protein found in immunoblots using sera from European patients who experienced anaphylaxis from *Chrysops* bites (33).

Sand Flies

Sand fly saliva contains a factor that enhances the infectivity of *Leishmania* by inhibiting the ability of interferon- γ to activate macrophages. It also reduces nitric oxide production (34). A delayed-type hypersensitivity reaction to saliva components may also play a role in infectivity and adverse reactions (35). Sand fly saliva is also known to contain a potent vasodilator named maxadilan, apyrase, 5'-nucleotidase, hyaluronidase. Other proteins include an anticlotting protein with a carbohydrate-recognition domain and several proteins of unknown function.

Kissing Bugs and Bedbugs

The major salivary anticoagulant proteins of *Rhodnius prolixus* are named prolixins and consist of four related nitrophorin molecules (36), which are heme proteins that carry nitric oxide. The major component has a molecular weight of 19,689 Da and inhibits factor VIII-mediated activation of factor X. Two proteins have been characterized from the saliva of *T. pallidipennis*, triabin of molecular weight 15,620 Da, an inhibitor of thrombin-based hydrolysis of fibrinogen (37), and pallidipin (38) of molecular weight 19,000 Da, an inhibitor of collagen-induced platelet aggregation. Functional studies of coagulation inhibition suggest that different species of Triatominae have functionally different mechanisms of coagulation inhibition and different sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of salivary proteins (39). These proteins along with proteins having histamine binding, platelet inhibition, anticoagulation, and nitric oxide transport activities are all members of the lipocalin family (40). The three-dimensional structures of the nitrophorins NP1, 2, and 4 have been determined by X-ray crystallography. Many important vertebrate-derived allergens are also members of the lipocalin family. An activatable serine protease of molecular weight 40,000 Da, named triapsin, with an arginine specificity, has been isolated from saliva of *T. infestans* (41).

Studies to characterize the allergenic proteins of *T. protracta* indicate that the major allergens are of molecular weight 18,000 to 20,000 Da and almost all the allergenic activity was found between pI 6.7 and 7.3 and at pI 8.2 (42). This allergen, a member of the lipocalin family named procalin, has been identified, cloned, and expressed in yeast cells (43). The recombinant procalin reacts in ELISA assays with IgE antibodies from allergic patients and cross-reacts with native allergen. Antiserum against procalin was used in immunohistochemistry to localize procalin to the cytoplasm of cuboidal epithelium and the luminal contents of the salivary glands.

The saliva of the bedbug, *C. lectularius*, contains a nitrophorin (44) and also an inhibitor of activation of factor X to factor Xa in the tenase complex, which does not directly inhibit factor VIII (45). The apparent molecular weight of this factor was 17,000 Da. Bedbug saliva also contains apyrase.

Fleas

Very little work has been done applying contemporary methods to studies of flea saliva. The only characterized proteins in flea saliva are apyrase, which prevents ADP-induced platelet aggregation (46), platelet-activating factor acetylhydrolase, and naphthyl esterases.

Diagnostic studies of allergy to flea bites in humans are complicated by the relatively more common occurrence of inhalant allergy to cat fleas. The major salivary allergen of cat fleas causing allergy in dogs is a protein of molecular weight 18,000 Da and pI 9.3, termed Cte f 1 (47).

Ticks

There has been a great deal of interest in ticks with the recognition of Lyme disease and ehrlichiosis. Allergic reactions to tick bites are usually the result of bites by soft ticks, Ixodidae. Pigeon ticks, *Argas reflexus*, as well as deer ticks and paralysis ticks have all been reported to cause systemic allergic reactions. Tick salivas have been found to contain apyrase and antiplatelet activities (13,48) as well as numerous proteins from 18 to 160 kDa. Several proteins from 15 to 50 kDa were induced by feeding (49).

Five salivary allergens have been isolated from the Australian paralysis tick, *Ixodes holocyclus*, of molecular weights 28, 45, 50, 55, and 355 kDa (50). The allergens at 28 and 355 kDa appear to react with IgE from most patients and SGA1 at 28 kDa is useful for skin prick testing and radioimmunoassay (51).

CROSS-REACTIVITY AMONG BITING INSECTS

There is very limited experimental data on IgE cross-reactivity among biting insects. There are some common antigens exhibiting a limited degree of cross-reactivity among mosquito genera and species (21-23). However, typical clinical reactions, including nonallergic responses, are species dependent for most individuals. There appears to be some cross-reactivity based on RAST testing between horseflies and deerflies and sometimes also blackflies (1). It is not known if this is clinically relevant. There are antigen 5-related proteins in insect salivas (11), which could be cross-reactive with vespid venom proteins.

Allergic reactions to kissing bugs and bedbugs exhibit a strong species dependence, and it is rare to find patients either skin test positive or RAST positive to more than a single species (52).

There are no data on cross-reactivity with fleas in human subjects, but studies on dogs suggest species specificity. Reactions to sand flies, biting midges, ticks, tsetse flies, and other biting arthropods are probably species specific, but experimental data are lacking.

BITING INSECT CONTROL

The control of biting insects is a very difficult problem, as attempts at mosquito vector control in the tropical world have demonstrated. Use of most pesticides, especially large area spraying, is best left to public health authorities. Spraying of yards is usually not recommended, is almost always of limited value, and may involve significant risk of pesticide exposure to children and pets.

Control of biting insects in the home should emphasize avoidance. Screens should be used on all doors and windows. Various forms of flypaper traps with and without attractants are effective and environment friendly. One highly recommended type is clear and is placed on glass doors and windows, another uses 7-W light bulbs. Control of fleas from pets, particularly in warm and humid areas, can be extremely difficult. Veterinarians can recommend several programs, including the use of growth regulators that are fed to dogs and cats to prevent development of adult fleas and substances that are spotted onto the animal

and absorbed through the skin or others that are injected. The extensive use of anti-acetylcholinesterase pesticides is ineffective and leads to development of resistant fleas. Animals should be regularly washed and carpets and furniture regularly vacuumed to help control fleas.

Bedbug infestations should be eliminated by treatment with appropriate pesticides, preferably by a licensed professional. Reduviid bugs are primarily outdoor insects and are best controlled by eliminating their definitive hosts around houses. *T. protracta* comes from wood rat nests, but other species have varied hosts. Professional assistance is recommended.

Horseflies, deerflies, and blackflies are primarily found around water. They can be extremely difficult to avoid in these areas. The almost ubiquitous mosquito is extremely difficult to avoid. The use of repellants containing DEET (*N,N'*-diethyl-*m*-toluamide) can help; these should be used with caution in small children. Many other repellants are less effective. Covering up of as much exposed skin as possible and avoiding being outdoors at high-risk times, like early morning and evening, can help. Avoidance of areas of high mosquito density should be practiced. Sources of standing water should be minimized or eliminated. Mosquito netting and the use of citronella candles can also reduce mosquito density. An ultraviolet bug light can also help, particularly after dark. Both electrocuting and trap models are available. Use of yellow or orange light bulbs outdoors minimizes the attraction of insects to porches and garages.

Reliable guidance can be obtained from websites sponsored by various government agencies: for example, <http://wwwn.cdc.gov/travel/yellowBookCh2-InsectsArthropods.aspx>, <http://www.epa.gov/pesticides/health/mosquitoes/insectrp.htm>, and <http://www.ces.ncsu.edu/depts/ent/notes/Urban/repellents.htm>.

IMMUNOTHERAPY

Evidence for Efficacy

There is very limited controlled study evidence for the efficacy of immunotherapy in preventing life-threatening systemic reactions to insect bites. There are a significant number of anecdotal reports, most of which describe variants of large local reactions. The only challenge verified trial with an insect salivary gland-derived vaccine was reported in a noncontrolled study with *T. protracta* in 1984 (53). Immunotherapy provided protection in all five patients with no significant side effects. Immunologic changes were also observed in parallel with protection as assessed by bite challenge.

A report of treatment with deerfly whole-body vaccine, although not controlled, suggests efficacy for patients with systemic reactions (54). Immunotherapy with whole-body extracts has been tried in cases of life-threatening allergy to mosquito bites (55). Results have been mixed with some patients developing the ability to tolerate a larger number of bites and others developing major complications as described below.

It should be noted that in the United States and most other countries, there are no licensed extracts of insect saliva or salivary glands and that most whole-body extracts from biting insects are not approved for use in allergen vaccine therapy. These products should only be used under an investigational new drug (IND) application, as part of a controlled study. One study (56) demonstrated that it is possible to prepare substantially more potent vaccines from biting insects than are available in current commercial products. In some countries, special unlicensed products can be prepared for individual patients.

Most cases of severe allergy to mosquito bites are best managed by prophylactic use of antihistamines such as cetirizine (57). In controlled trials, cetirizine has been shown to reduce pruritus, significantly decrease large local reaction development, and may also prevent systemic reactions (57,58). The antihistamine, loratadine, used prophylactically reduces whealing and pruritis from mosquito bites in children; it also reduces the size of bite lesions at 24 hours (59).

Known Risks

Immunotherapy with mosquito whole-body vaccine has been shown to cause local pain, swelling, and redness in a patient who tolerated injections at lower concentrations. Another patient in the same report developed arthralgias, fatigue, myalgias, weakness and swelling of

distal extremities, despite treatment with terfenadine, cimetidine, and prednisone (55). Life-threatening anaphylactic reactions have been observed in studies of experimental vaccines derived from mosquito cell tissue culture (60).

The use of other biting insect vaccines has not been reported to cause unusual reactions, and the experiences reported in the literature correspond to those seen with other allergens routinely used in allergen vaccine therapy.

Potential Risks

The existence of species and genus specificity for many biting insect reactions requires the use of more sophisticated diagnostic reagents than are currently commercially available. There is a significant risk of using an ineffective preparation and a potential risk of sensitization. Also, many hematophagous insects are vectors for serious diseases (parasitic, viral, rickettsial, and bacterial). Extracts prepared from salivary glands must be carefully monitored to be free of infectious agents. The use of biting insect extracts in allergen vaccine therapy is, for the most part, an experimental procedure, and proper safety procedures and regulations are necessary.

MOST IMPORTANT AND SALIENT POINTS

- There are a large variety of hematophagous insects and arachnids.
- Many different arthropods can cause bite allergy.
- Many, if not most, insect bite allergies may be species and/or genus specific.
- Insect saliva varies widely, but most species contain potent anticoagulants and digestive enzymes.
- The best diagnostic reagents are insect saliva or salivary gland extract, but none are commercially available or licensed in the United States or in Europe.
- Immunotherapy has been shown to be effective prophylaxis for severe systemic reactions for *T. protracta*, deerflies, and mosquitoes in uncontrolled studies.
- Immunotherapy with mosquito whole-body vaccine has been associated with significant side effects.
- Immunotherapy with biting insect whole body, salivary gland, and saliva extracts is still, for the most part, an experimental procedure.
- Control of many biting insects is difficult, but risk of exposure to bites can be greatly reduced.
- Reactions to mosquito bites are best managed by prophylaxis with cetirizine or loratadine.

REFERENCES

1. Hoffman DR. Allergic reactions to biting insects. In: Levine MI, Lockey RF, eds. Monograph on Insect Allergy. 4th ed. Milwaukee: American Academy of Allergy, Asthma and Immunology, 2003:161-173.
2. Van Wye JE, Hsu Y-P, Lane RS, et al. IgE antibodies in tick bite induced anaphylaxis. *J Allergy Clin Immunol* 1991; 88:968-970.
3. Gauci M, Loh RKS, Stone BF, et al. Allergic reactions to the Australian paralysis tick, *Ixodes holocyclus*. Diagnostic evaluation by skin test and radioimmunoassay. *Clin Exp Allergy* 1989; 74:279-283.
4. Arnett RH. American Insects. New York: Van Nostrand Reinhold, 1985.
5. Ribeiro JM. Blood-feeding arthropods: live syringes or invertebrate pharmacologists? *Infect Agents Dis* 1995; 3:143-152.
6. Tabachnik WJ. Pharmacological factors in the saliva of blood-feeding insects. *Ann N Y Acad Sci* 2000; 916:444-452.
7. Kerlin RL, Hughes S. Enzymes in saliva from four parasitic arthropods. *Med Vet Entomol* 1992; 6:121-126.
8. Theodos CM, Ribeiro JM, Titus RG. Analysis of enhancing effect of sand fly saliva on *Leishmania* infection in mice. *Infect Immun* 1991; 59:1592-1598.
9. Donovan MJ, Messmore AS, Scrafford DA, et al. Uninfected mosquito bites confer protection against infection with malaria parasites. *Infect Immun* 2007; 75:2523-2530.
10. Holt RA, Subramanian GM, Halpern A, et al. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* 2002; 298:129-149.

11. Ribeiro JM, Adams J. Allergic reactions to mosquito bites. *Adjuvants* 1991; 1:1-10.
12. Ribeiro JM, Adams J. Allergic reactions to mosquito bites. *Rheumatology* 1991; 30:1-10.
13. Vale J, Adams J. Allergic reactions to mosquito bites. *J Exp Med* 1991; 173:1-10.
14. Ribeiro JM, Adams J. Allergic reactions to mosquito bites. *gammaglobulin* 1991; 1:1-10.
15. Chakrabarti S, Adams J. Allergic reactions to mosquito bites. *Aedes* 1991; 1:1-10.
16. James J. Allergic reactions to mosquito bites. *mosquito* 1991; 1:1-10.
17. Argente J. Allergic reactions to mosquito bites. *Aedes* 1991; 1:1-10.
18. Starke J. Allergic reactions to mosquito bites. *mosquito* 1991; 1:1-10.
19. James J. Allergic reactions to mosquito bites. *majors* 1991; 1:1-10.
20. Peng J. Allergic reactions to mosquito bites. *salivary gland* 1991; 1:1-10.
21. Peng J. Allergic reactions to mosquito bites. *mosquito* 1991; 1:1-10.
22. Li H. Allergic reactions to mosquito bites. *world* 1991; 1:1-10.
23. Peng J. Allergic reactions to mosquito bites. *three* 1991; 1:1-10.
24. Xu V. Allergic reactions to mosquito bites. *2, and* 1991; 1:1-10.
25. Bruner J. Allergic reactions to mosquito bites. *22kD* 1991; 1:1-10.
26. Bruner J. Allergic reactions to mosquito bites. *aegypti* 1991; 1:1-10.
27. Cupo J. Allergic reactions to mosquito bites. *Hygiene* 1991; 1:1-10.
28. Cupo J. Allergic reactions to mosquito bites. *pote* 1991; 1:1-10.
29. Wirtz R. Allergic reactions to mosquito bites. *Simu* 1991; 1:1-10.
30. Farkas J. Allergic reactions to mosquito bites. *bites* 1991; 1:1-10.
31. Grev J. Allergic reactions to mosquito bites. *inhib* 1991; 1:1-10.
32. Reddy J. Allergic reactions to mosquito bites. *recep* 1991; 1:1-10.
33. Hemm J. Allergic reactions to mosquito bites. *IgE-l* 1991; 1:1-10.
34. Hall J. Allergic reactions to mosquito bites. *killin* 1991; 1:1-10.
35. Belk J. Allergic reactions to mosquito bites. *sand* 1991; 1:1-10.
36. Chan J. Allergic reactions to mosquito bites. *nitric* 1991; 1:1-10.
37. Noes J. Allergic reactions to mosquito bites. *J Bio* 1991; 1:1-10.

11. Ribeiro JMC, Arca B, Lombardo F, et al. An annotated catalogue of salivary gland transcripts in the adult female mosquito, *Aedes aegypti*. BMC Genomics 2007; 8:6.
12. Ribeiro JMC, Anderson J, Silva-Neto MAC, et al. Exploring the sialome of the blood-sucking bug, *Rhodnius prolixus*. Insect Biochem Mol Biol 2004; 34:61-79.
13. Valenzuela JG, Francischetti IM, Pham VM, et al. Exploring the sialome of the tick *Ixodes scapularis*. J Exp Biol 2002; 205:2843-2864.
14. Ribeiro JM, Nussenzveig RH, Tortorella G. Salivary vasodilators of *Aedes triseriatus* and *Anopheles gambiae* (Diptera: Culicidae) J Med Entomol 1994; 31:747-753.
15. Champagne DE, Smartt CT, Ribeiro JM, et al. The salivary gland-specific apyrase of the mosquito *Aedes aegypti* is a member of the 5'-nucleotidase family. Proc Natl Acad Sci U S A 1995; 92:694-698.
16. James AA, Blackmer K, Racioppi JV. A salivary gland-specific, maltase-like gene of the vector mosquito, *Aedes aegypti*. Gene 1989; 75:73-83.
17. Argentine JA, James AA. Characterization of a salivary gland-specific esterase in the vector mosquito, *Aedes aegypti*. Insect Biochem Mol Biol 1995; 25:621-630.
18. Stark KR, James AA. A factor Xa-directed anticoagulant from the salivary glands of the yellow fever mosquito *Aedes aegypti*. Exp Parasitol 1995; 81:321-331.
19. James AA, Blackmer K, Marinotti O, et al. Isolation and characterization of the gene expressing the major salivary gland protein of the female mosquito, *Aedes aegypti*. Mol Biochem Parasitol 1991; 44:245-253.
20. Peng Z, Beckett AN, Engler RJ, Hoffman DR, Ott NL, Simons FER. Immune responses to mosquito saliva in 14 individuals with systemic allergic reactions to mosquito bites. J. Allergy Clin. Immunol. 2004; 114:1189-94.
21. Peng Z, Li HB, Simon FER. Immunoblot analysis of IgE and IgG binding antigens in extracts of mosquitoes *Aedes vexans*, *Culex tarsalis* and *Culiseta inornata*. Int Arch Allergy Immunol 1996; 110:46-51.
22. Li H, Simons FER, Peng Z. Immunoblot analysis of salivary allergens in 10 mosquito species with worldwide distribution and the human IgE responses to these allergens. J Allergy Clin Immunol 1998; 101:498-505.
23. Peng Z, Simons FE. Cross-reactivity of skin and serum specific IgE responses and allergen analysis for three mosquito species with worldwide distribution. J Allergy Clin Immunol 1997; 100:192-198.
24. Xu W, Lam H, Peng Z, et al. Mosquito allergy: expression, purification and characterization of Aed a 2, an *Aedes aegypti* salivary allergen. J Allergy Clin Immunol 1997; 99:S152 (abstr).
25. Brummer-Korvenkontio H, Kalkkinen N, Palusuo T, et al. Molecular characterization of the major 22kD *Aedes communis* mosquito saliva allergen. J Allergy Clin Immunol 1997; 99:S353 (abstr).
26. Brummer-Korvenkontio H, Palusuo T, Francois G, et al. Characterization of *Aedes communis*, *Aedes aegypti* and *Anopheles stephensi* mosquito saliva antigens by immunoblotting. Int Arch Allergy Immunol 1997; 112:169-174.
27. Cupp MS, Ribeiro JM, Cupp EW. Vasodilative activity in black fly salivary glands. Am J Trop Med Hyg 1994; 50:241-246.
28. Cupp MS, Ribeiro JMC, Champagne DE, et al. Analyses of cDNA and recombinant protein for a potent vasoactive protein in saliva of a blood-feeding fly, *Simulium vittatum*. J Exp Biol 1998; 201:1553-1561.
29. Wirtz HP. Bioamines and proteins in the saliva and salivary glands of palaearctic blackflies (Diptera: Simuliidae). Trop Med Parasitol 1990; 41:59-64.
30. Farkas J. Simuliosis. Analysis of dermatological manifestations following blackfly (Simuliidae) bites as observed in the years 1981-1983 in Bratislava (Czechoslovakia). Derm Beruf Umwelt 1984; 32: 171-173.
31. Grevelink SA, Youssef DE, Loscalzo J, et al. Salivary gland extracts from the deerfly contain a potent inhibitor of platelet aggregation. Proc Natl Acad Sci U S A 1993; 90:9155-9158.
32. Reddy VB, Kounga K, Mariano F, et al. Chrysoptin is a potent glycoprotein IIB/IIIa fibrinogen receptor antagonist present in salivary gland extracts of the deerfly. J Biol Chem 2000; 275:15861-15867.
33. Hemmer W, Focke M, Vieluf D, et al. Anaphylaxis induced by horsefly bites: identification of a 69kd IgE-binding salivary gland protein from *Chrysops* spp. (Diptera, Tabanidae) by western blot analysis. J Allergy Clin Immunol 1998; 101:134-136.
34. Hall LR, Titus RG. Sand fly vector saliva selectively modulates macrophage functions that inhibit killing of *Leishmania major* and nitric oxide production. J Immunol 1995; 155:3501-3506.
35. Belkaid Y, Valenzuela JG, Kamhawi S, et al. Delayed-type hypersensitivity to *Phlebotomus papatasi* sand fly bite: an adaptive response induced by the fly? Proc Natl Acad Sci U S A 2000; 97:6704-6709.
36. Champagne DE, Nussenzveig RH, Ribeiro JM. Purification, partial characterization, and cloning of nitric oxide-carrying heme proteins (nitrophorins) from salivary glands of the blood sucking insect *Rhodnius prolixus*. J Biol Chem 1995; 270:8691-8695.
37. Noeske-Jungblut C, Haendler B, Donner P, et al. Triabin, a highly potent exosite inhibitor of thrombin. J Biol Chem 1995; 270:28629-28634.

38. Haendler B, Becker A, Noeske-Jungblut C, et al. Expression, purification and characterisation of recombinant pallidipin, a novel platelet aggregation inhibitor from the hematophagous triatome bug *Triatoma pallidipennis*. *Blood Coagul Fibrinolysis* 1996; 7:183-186.
39. Pereira MH, Souza ME, Vargas AP, et al. Anticoagulant activity of *Triatoma infestans* and *Panstrongylus megistus* saliva (Hemiptera/Triatominae). *Acta Trop* 1996; 61:255-261.
40. Montfort WR, Weichsel A, Anderson JF. Nitrophorins and related antihemostatic lipocalins from *Rhodnius prolixus* and other blood-sucking arthropods. *Biochim Biophys Acta* 2000; 1482:110-118.
41. Amino R, Tanaka AS, Schenkman S. Triapsin an unusual activatable serine protease from the saliva of the hematophagous vector of Chagas' disease *Triatoma infestans* (Hemiptera: Reduviidae). *Insect Biochem Mol Biol* 2001; 31:465-472.
42. Chapman MD, Marshall NA, Saxon A. Identification and partial purification of species-specific allergens from *Triatoma protracta* (Heteroptera:Reduviidae). *J Allergy Clin Immunol* 1986; 78:436-442.
43. Paddock CD, McKerrow JH, Hansell E, et al. Identification, cloning and recombinant expression of procalin, a major triatomine allergen. *J Immunol* 2001; 167:2694-2699.
44. Valenzuela JG, Walker FA, Ribeiro JM. A salivary nitrophorin (nitric-oxide-carrying hemoprotein) in the bedbug *Cimex lectularius*. *J Exp Biol* 1995; 198:1519-1526.
45. Valenzuela JG, Guimaraes JA, Ribeiro JM. A novel inhibitor of factor X activation from the salivary glands of the bed bug *Cimex lectularius*. *Exp Parasitol* 1996; 83:184-190.
46. Ribeiro JM, Vaughn JA, Azad AF. Characterization of the salivary apyrase activity of three rodent flea species. *Comp Biochem Physiol B* 1990; 95:215-219.
47. McDermott MJ, Weber E, Hunter S, et al. Identification, cloning, and characterization of a major cat flea salivary allergen (Cte f 1). *Mol Immunol* 2000; 37:361-375.
48. Ribeiro JM, Endris TM, Endris R. Saliva of the soft tick, *Ornithodoros moubata*, contains anti-platelet and apyrase activities. *Comp Biochem Physiol A* 1991; 100:109-112.
49. Sanders ML, Scott AL, Glass GE, et al. Salivary gland changes and host antibody responses associated with feeding of male lone star ticks (Acari:Ixodidae). *J Med Entomol* 1996; 33:628-634.
50. Gauci M, Stone BF, Thong YH. Isolation and immunological characterization of allergens from salivary glands of the Australian paralysis tick *Ixodes holocyclus*. *Int Arch Allergy Appl Immunol* 1988; 87:208-212.
51. Gauci M, Loh RKS, Stone BF, et al. Evaluation of partially purified salivary gland allergens from the Australian paralysis tick, *Ixodes holocyclus* in diagnosis of allergy by RIA and skin prick test. *Ann Allergy* 1990; 64:297-299.
52. Marshall NA, Chapman MD, Saxon A. Species specific allergens from the salivary glands of Triatominae (Heteroptera:Reduviidae). *J Allergy Clin Immunol* 1986; 78:430-435.
53. Rohr AS, Marshall NA, Saxon A. Successful immunotherapy for *Triatoma protracta*-induced anaphylaxis. *J Allergy Clin Immunol* 1984; 73:369-375.
54. Wilbur RD, Evans R. An immunologic evaluation of deerfly hypersensitivity. *J Allergy Clin Immunol* 1975; 55:72.
55. McCormack DR, Salata KF, Hershey JN, et al. Mosquito bite anaphylaxis: immunotherapy with whole body extracts. *Ann Allergy Asthma Immunol* 1995; 74:39-44.
56. Peng ZK, Simon FER. Comparison of proteins, IgE and IgG binding antigens, and skin reactivity in commercial and laboratory-made mosquito extracts. *Ann Allergy Asthma Immunol* 1996; 77:371-376.
57. Reunala T, Brummer-Korvenkontio H, Karppinen A, et al. Treatment of mosquito bites with cetirizine. *Clin Exp Allergy* 1993; 23:72-75.
58. Karppinen A, Rantala I, Vaalasti A, et al. Effect of cetirizine on the inflammatory cells in mosquito bites. *Clin Exp Allergy* 1996; 26:703-709.
59. Karppinen A, Kautianen H, Reunala T, et al. Loratadine in the treatment of mosquito-bite-sensitive children. *Allergy* 2000; 55:668-671.
60. Scott RM, Shelton AL, Eckels KH, et al. Human hypersensitivity to a sham vaccine prepared from mosquito cell culture fluids. *J Allergy Clin Immunol* 1984; 74:808-811.

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