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ROLE OF ARTHROPOD SALIVA IN BLOOD FEEDING: Sialome and Post-Sialome Perspectives*

José M. C. Ribeiro and Ivo M. B. Francischetti
Medical Entomology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 4 Center Drive, Bethesda, Maryland, 20892-0425; e-mail: Jribeiro@nih.gov; Ifrancischetti@niaid.nih.gov

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Key Words hemostasis, inflammation, nociception, mast cells, salivary glands

■ **Abstract** This review addresses the problems insects and ticks face to feed on blood and the solutions these invertebrates engender to overcome these obstacles, including a sophisticated salivary cocktail of potent pharmacologic compounds. Recent advances in transcriptome and proteome research allow an unprecedented insight into the complexity of these compounds, indicating that their molecular diversity as well as the diversity of their targets is still larger than previously thought.

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INTRODUCTION

Fifteen years ago it was proposed that (71), based on enzymatic and bioassay data, saliva of bloodsucking arthropods served mainly an antihemostatic role and, additionally, that hard tick saliva served to evade their hosts' inflammation and immunity [a concept more detailed in (72)]. Except for tick salivary prostaglandins (25, 34), no other hematophagous arthropod salivary compound had been molecularly characterized at that time. Thanks to the revolutionary techniques of molecular biology as well as the miniaturization of high-performance liquid chromatography, mass spectrometry, and Edman degradation techniques, detailed knowledge of such molecules and their role in blood feeding has increased at a fast pace. Accordingly, it was confirmed that almost all bloodsucking arthropods studied (a small sample consisting of only a few species from over 500 genera and ~19,000 known species) (73) have at least one anticlotting, one vasodilator, and one antiplatelet compound. However, the molecular diversity of the nature of such compounds was very large. We have also learned that hematophagous insects and ticks have many different and sometimes apparently conflicting salivary strategies, which were not predicted before. In addition, at least one half of the message expressed in the salivary glands of such animals, leading to apparently secreted proteins, has no known function, thus challenging the researchers' knowledge and imagination. The reader unfamiliar with saliva of vector arthropods is encouraged to read previous reviews (11, 73). Several excellent reviews have been written on the role of saliva in host immunity and parasite transmission (31, 87, 100, 101).

WHY IT IS DIFFICULT TO STEAL BLOOD FROM A VERTEBRATE

To a bloodsucking animal, paradise is a place where the host blood does not clot, the blood flow at the feeding site is intense, and the host will not bother (or kill) the guest. Real life is different. Vertebrates have three efficient systems that make life potentially difficult for hematophagous animals: hemostasis, inflammation, and immunity. These three complex physiological responses interact with each other and, at times, are in opposition.

Hemostasis

Hemostasis is the host response that controls the loss of blood following injury to a blood vessel. It consists of platelet aggregation, blood coagulation, and vasoconstriction. All these phenomena are redundant. There are several independent agonists of platelet aggregation [ADP, collagen, thrombin, platelet-activating factor, thromboxane A_2 (TXA_2)], at least two vasoconstrictors are released by platelets (TXA_2 and serotonin), and the clotting cascade is a complex system with many potential points of amplification and control. For more details on hemostasis see References 11, 73. Hemostasis thus takes care of the host blood loss following injury and places a major barrier to any blood-feeding arthropod.

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Inflammation

Inflammation is the host response following tissue injury. Classically, it consists of the triple response of Lewis: pain, redness, and heat; the last two last being the result of tissue vasodilatation. Although vasodilatation is favorable to blood feeding, pain triggers the host's awareness to the blood sucker. To ticks, tissue repair may lead to encapsulation and isolation of the feeding mouthparts from live tissue. An aseptic tissue injury produces a series of events leading to tissue repair. ATP, released by injured cells, is also responsive to the immediate and acute pain following tissue injury (16). Serotonin and histamine, released by platelets and mast cells, are also inducers of pain and increased vascular permeability, as is bradykinin, which is produced following activation of factor XII by tissue-exposed collagen (38). Activated factor XII converts prekallikrein to kallikrein, which hydrolyzes blood kininogen to produce the vasodilatory peptide, bradykinin. Note that many of the hemostasis mediators are linked to pain production in inflammation.

Polymorphonuclear cells and monocytes are important mediators of inflammation. ATP, released by injured cells, activates neutrophils that accumulate and degranulate at the injury site (46, 58). Thrombin from the blood-coagulation cascade and other proinflammatory molecules, such as platelet-activating factor, also activate neutrophils that produce prostaglandins and platelet-activating factor itself (8, 44). Neutrophil activation is also accompanied by the release of several proteases modulating platelet function, such as cathepsin G (82), or enzymes that act on the tissue matrix, such as elastase (33, 80, 85). Importantly, thrombin also has inflammatory properties including causing fibroblast proliferation and increasing neutrophil adhesion, whereas clotting factor Xa functions as mediator of acute inflammation by binding to effector cell protease receptor-1, inducing vascular permeability and leukocyte exudation (18, 32, 52). Activation of neutrophil and other cell types is also accompanied by generation of pain-inducing prostaglandins (38). Pain is also induced by chemokines (inflammatory proteins) such as interleukin-1 (IL-1) generated by neutrophils, as well as bradykinin produced by the intrinsic pathway of blood coagulation. Therefore, several molecules work in concerted manner to generate pain (55). In this regard, bradykinin induces TNF-α release from neutrophils (27, 61), which in turn stimulates the release of IL-1β and IL-6 from various cell types including those of the phagocyte mononuclear system. These cytokines contribute to the phenomenon of increased sensitivity to pain, or hyperalgesia, that accompanies inflammation. Cytokine-mediated inflammatory hyperalgesia is accompanied by production of cyclo-oxygenase products and IL-8 released by monocytes, macrophages, and endothelial cells, which stimulate the production of sympathomimetic mediators also involved in increased pain reception (19, 20).

In the case of septic injury to the tissue, the inflammatory response is amplified. Activation of the alternative or colectin complement pathway by bacterial or fungal surfaces leads to the production of anaphylatoxins, which are potent molecules attracting granulocytes and monocytes to the injury site (40, 99). Bacterial lipopolysaccharide also induces activation of various leukocytes, leading,

for example, monocytes, neutrophils, and eosinophils to produce vasodilatory prostaglandins, various cytokines, superoxide, and nitric oxide, as well as releasing their granules (39, 50, 62, 88, 103). Both macrophages and neutrophils actively phagocytose bacteria, and their products influence each other (80).

Following this acute phase, the resultant inflammatory, complement, and hemostatic reactions produce a unique environment within wounds that promotes repair. Repair involves four components: angiogenesis (formation of new blood vessels), migration and proliferation of fibroblasts (fibroplasia), deposition of extracellular matrix, and remodeling. Repair begins early in inflammation, sometimes as early as 24 h after injury. Remarkably, endothelial cell proliferation is an indispensable early process in the formation of new blood vessels, and it is fundamental to tissue repair because blood vessels carry oxygen and nutrients necessary to sustain cell metabolism (104). Other cell types are also involved in repair mechanisms. Macrophages provide a continuing source of cytokines necessary to stimulate fibroplasia and angiogenesis, whereas fibroblasts construct new extracellular matrix necessary to support cell growth. In this process, proliferative responses are triggered by a number of growth factors released by endothelial cells, platelets, macrophages, and fibroblasts (89). Because some of these cell types are activated by enzymes of the blood-coagulation cascade (e.g., thrombin), angiogenesis and hemostasis are interrelated (7). Accordingly, platelet aggregation and blood-coagulation inhibitors found in the salivary gland of blood feeders may also negatively modulate angiogenesis in vivo. However, for most fast feeders such as mosquitoes, sand flies, triatomines, and fleas, tissue repair is not a major barrier as the events are on a timescale of hours and days, whereas feeding takes minutes. On the other hand, hard ticks, which feed continuously for 3–10 days with their mouthparts imbedded in their hosts, face host-repair mechanisms that may get in the way of a satisfactory meal.

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Immunity

Exposure of foreign antigens to a vertebrate in an inflammatory context leads the immune system to further recognize these molecules and react accordingly. The human immune response to mosquito bites was described by Mellanby (54) as progressing from no reaction, to delayed-type hypersensitivity (DTH), to immediate-type hypersensitivity, and to desensitization. Exposure of skin antigens to naïve animals leads dendritic cells to initially process the antigen, and then to activate and clonally expand the so-called DTH–T cells. After this T cell expansion, and following new antigen deposition in the skin, these circulating cells congregate in the vicinity of the antigen and start producing various inflammatory cytokines, including γ-interferon, which activates local macrophages to produce TNF-α and IL-1, two mediators of inflammatory pain (26, 27). Because some of these cells are not in the skin and have to accumulate from the blood circulation, it takes 6–12 h for the monocytic infiltrate to be barely noticeable, and they achieve peak infiltration at 24-h postdermal antigen exposure, thus the name DTH. We may

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speculate that the vertebrate perception of the DTH induces the vertebrates to avoid behaviorally the re-exposure to the source of the nuisance. The DTH may actually be of advantage to some sand flies, and to nest-associated blood feeders such as *Cimex* and fleas, as proposed before (4). To ticks, a special type of DTH, the basophil infiltrate characterizing the Motte Jones response (2), is associated with tick-rejection reactions (1, 6), especially in guinea pig models. The rejection occurs by a behaviorally defensive action of the host (scratching), as well as by disturbance of feeding, wherein blood is substituted by a purulent infiltrate. These leukocytes, particularly eosinophils, may also prove noxious by acting on the tick gut (101, 102).

Following continuous exposure of the antigen, a new subset of lymphocytes is activated, leading to the production of IgE by a B-cell subtype. Mast cells, loaded with histamine (or histamine and serotonin, depending on the vertebrate species) and residing on the connective tissue such as the dermis, have high-affinity receptors to IgE (35). When divalent antigens cross-link two IgE bound to the mast cell IgE receptor, the cell degranulates, releasing their vasoactive amines. After activation, mast cells also produce and release several arachidonic acid metabolites and a diversity of cytokines, including IL-4, which stimulates the immune response to progress toward a Th2- or antibody-mediated response. Mast cells also produce nerve-growth factor, which acts on nociceptive neurons to decrease their threshold to pain-inducing molecules such as bradykinin, serotonin, and histamine (38). Histamine promotes more vasodilatation in the arteriolar side than in the venular side of the skin circulation, thus creating an increase in the hydrostatic pressure of the capillaries. Histamine (as well as the aforementioned inflammatory substances serotonin and bradykinin) also increases the spacing of the endothelial cells, which, together with the larger capillary hydrostatic pressure, leads to extravasation of plasma into the interstitial tissue, creating edema. These vasoactive substances are quick acting, producing a visible response within a minute or two of their release, but they do not last long because they are washed out of the tissue or metabolized. After 20–30 min, most of the reaction is gone. To the vertebrate host, this reaction leads to a behavioral response leading to the identification and removal of the annoying bloodsucker and, if possible, avoidance of the area of exposure. The host may also display life-threatening anaphylaxis response at this stage of its immune response to the arthropod bite. To the bloodsucker, this response may result in hunger, if it is lucky enough to escape, or death.

Continuation of the antigenic exposure to the vertebrate leads to maturation of the immune response into different subtypes of IgG. Although in humans IgG_4 binds to mast cell receptors (with much less affinity than IgE), other immunoglobulin types recognize the antigen, and the complex will likely be taken by macrophages that then digest these molecules—and possibly present them to lymphocytes—further maturing (i.e., increasing antibody specificity and affinity) the IgG response. The tissue response following antigen encounter within this immune response state is minimal, causing this stage of the reaction to an arthropod bite to be somewhat misleadingly named the desensitization stage. However, if the

antigen happens to bind to host skin cells, it may lead to complement fixation and tissue necrosis [the result of an Arthus reaction (15)]—a rare outcome to insect bites described in some types of allergy to triatomine bites (17). To the vertebrate host, this stage of the immune response (without complement fixation) leads to minimal annoyance. To the insect or tick, this stage may result in the neutralization of several molecules it uses in the feeding process.

In reality, these stages of the immune response may not be distinguished in a clear-cut way. It is common to have both a DTH and an immediate response, and to have a DTH and specific IgG antibodies simultaneously (a mixed-type response). Even in the presence of a mature response, a less intense but noticeable, immediate response may occur due to IgG4 (of human) or IgG1 (in guinea pigs) binding to mast cells. With all these mechanisms operating against them, how do bloodsuckers succeed?

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THE SALIVARY PHARMACOLOGIC COMPLEXITY OF HEMATOPHAGOUS ARTHROPODS

Perhaps because disarming a complex and redundant system such as hemostasis with a magic bullet is impossible, saliva of bloodsucking animals evolved a "magic potion," allowing them to succeed against all the complex barriers imposed by their hosts. As a rule, these animals' saliva contains at least one anticlotting, one antiplatelet, and one vasodilatory substance. In many cases, more than one molecule exists in each category. In some, compounds such as adenosine and nitric oxide that are at once antiplatelet and vasodilatory are found in saliva. It also became clear that, although only a few of the $>15{,}000$ species of >500 genera were studied (73), an enormous diversity of concoctions exist in these magic potions. The few vasodilators known thus far offer a good example of this diversity: The triatomine bug *Rhodnius prolixus* makes use of nitric oxide, as does the cimicid bug *Cimex lectularius* (76, 98). Because nitric oxide is an unstable gas, each bug developed a different heme protein that stabilizes and carries this gas to the host. *Rhodnius* nitrophorin is a member of the lipocalin family (9) and *Cimex* nitrophorin is a member of the inositol phosphatase family (97). Ticks have salivary prostaglandins in large amounts, including PGE_2 and PGF_2 (25, 34, 75). Old World sand flies of the genus *Phlebotomus* have adenosine as vasodilators (66, 68), but New World sand flies of the genus *Lutzomyia* have a 6.5-kDa peptide, maxadilan, the most potent vasodilator known, acting on PACAP receptors (48, 56). Note that *Phlebotomus* flies do not have maxadilan and *Lutzomyia* do not have adenosine in their saliva. The black fly *Simulium vittatum* has a 15-kDa vasodilator that acts on ATP-dependent K-channels (22, 23). This vasodilatory protein has no similarity to other known proteins. Finally, *Aedes* mosquitoes have a vasodilatory tachykinin decapeptide named sialokinin (10), while *Anopheles* mosquitoes have a vasodilatory peroxidase, of $\sim$65 kDa, which destroys skin-vasoconstricting norepinephrine and serotonin (78, 95). Accordingly, the salivary

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vasodilatory catalog goes from NO and prostaglandins to peptides up to a 65-kDa protein. Note also, members of the same insect family but not the same genus, such as the *Lutzomyia* and *Phlebotomus* or *Aedes* and *Anopheles*, have completely different vasodilators. The same diversity can be found for salivary anticlotting peptides. For example, *Aedes* has a salivary inhibitor of factor Xa that is a member of the serpin family (84), while *Anopheles* has a smaller antithrombin peptide unrelated to other known peptides (28, 95). A ubiquitous salivary enzyme is apyrase, which hydrolyzes both ATP and ADP to AMP, thus having an anti-pain, anti-inflammatory and antihemostatic activity (74). However, at least two different families of this enzyme exist. Mosquito apyrases are members of the 5′ nucleotidase family (12). In bacteria, these enzymes hydrolyze ATP, ADP, and AMP to adenosine and orthophosphate (105), while sand fly and the bed bug *Cimex* have an enzyme of a novel protein family (14, 91, 92). Other different anticlotting peptides exist and diversity in antiplatelet compounds was also found, which will not be discussed here. The picture of bloodsucker saliva that emerges is one of very diverse composition. To help understand the origins of this diversity, it is useful to consider that *Aedes* and *Anopheles* diverged more than 150 million years ago (63), or 100 million years before the radiation of mammals, while *Lutzomyia* and *Phlebotomus* diverged before the last tectonic plate separation, thus at a time coinciding with or prior to mammal radiation. We accordingly postulate that all genera that diverged before mammal radiation have considerable variation in their salivary composition.

It is becoming apparent that the salivary cocktail of hematophagous animals contains many substances that counteract host pain. True anesthetic substances inhibit nerve conduction, while substances that inhibit the action of pain agonists (nociceptive agents) have analgesic effects. The saliva of the bug *Triatoma infestans* inhibits sodium channel activity in nerves by an unspecified molecule (24), and this report contains the only account of such activity in arthropod saliva. Salivary components with potential antinociceptive effects are varied, including apyrase (by destroying ATP), histamine, and serotonin-binding proteins, thus far found in ticks and triatomine bugs (59, 70), and kininase, which destroys bradykinin (67). Active search of salivary components acting on nerve conduction and on mast cells should yield many more activities and the possible discovery of novel compounds.

Because ticks stay attached for several days on their hosts, they have potent anti-inflammatory and immunomodullatory components that may prevent or retard deleterious host responses or counteract pharmacologically their host's immunopharmacologic mediators. These include anticomplement (94), anti-IL-2 (30), protease inhibitors (47), antineutrophil activity (79), and other immunomodullatory molecules with unclear mechanism of action (5). Salivary gland extracts, or tick feeding, modify host cytokine expression in several ways (3, 41–43, 45, 51, 53). Considering the redundancy and complexity of the vertebrate immune system, individual tick species must have a complex immunosupressant cocktail, as is the case with their salivary antihemostatic cocktails. A challenge for the next few years will be to determine the extent and redundancy of such cocktails.

Ticks also contain salivary compounds that affect tissue repair. Notably, calreticulin, a molecule with potent antiangiogenesis properties and recently identified in the supernatants of Epstein-Barr virus–immortalized cells (60), has also been characterized in the salivary gland of the tick *Amblyomma americanum* (36). We have also reported the presence of the N terminus of calreticulin in the SDS/PAGE of *Ixodes scapularis* saliva (94a), and a potent inhibitory activity of both endothelial cell proliferation and chick aorta sprouting formation has been demonstrated in *I. scapularis* saliva (I.M.B. Francischetti & J.M.C. Ribeiro, unpublished observation).

The face fly *Haematobia irritans*, which is a cattle feeder, provides an interesting exception to the rule of a magic potion in saliva. Only a salivary anticlotting substance was found in this fly. We failed to find vasodilatory or antiplatelet activity (21); however, this fly feeds sparingly several times a day, taking a few microliters or less of blood at a time. Most of the time it stays attached to the cattle's face, where it feeds. We propose that this fly and possibly also the stable fly *Stomoxys* are in their evolutionary infancy of adaptation to blood feeding. These flies have an ancestor in common with house flies, leading to preadaptations such as long and robust mouthparts and a robust body. Their feeding apparatus is coarse when compared with the fine tools of a mosquito or a tsetse. They inflict quite a severe injury, with consequent pain, while feeding. Against this negative effect of feeding, they behaviorally found a place to feed where the host cannot do much about it. The cattle's tail or tongue cannot reach the flies, despite severe annoyance to the animal. Nor can movements of the dermal musculature have an impact. One day, millions of years from now, these flies may feed as well as their tsetse relatives, assuming their hosts will continue to be around.

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SALIVARY TRANSCRIPTOMES AND PROTEOMES (SIALOMES)

In the past three years it has become practically feasible to sequence full-length cDNA libraries obtained from the salivary glands of bloodsucking arthropods. Initially, we attempted a PCR-based subtractive hybridization protocol to enrich salivary-specific molecules (14). However, these protocols usually led to obtaining fragmentary, not full-length sequences, owing to the use of restriction enzymes in the manufacture of the library. By mass sequencing nonsubtracted full-length sequence libraries, we obtained the same information as in the subtracted library, with the bonus of being much easier to obtain full-length sequence information by RACE (rapid amplification of cDNA ends) protocols (96). By using PCR-based techniques to construct these libraries, as few as 20–50 pairs of glands from mosquitoes or sand flies yield complex libraries. By sequencing 500–1000 clones of each library, and clustering the cDNA sequences by their similarity, we identified almost all or most of the previously reported sequences of proteins or peptides from salivary glands of hematophagous arthropods deposited in GenBank, and many more. For example, in a cDNA library of the mosquito *Aedes aegypti*, we found

all the six previously deposited sequences and described 30 new ones (96). In the sand fly *Phlebotomus papatasi*, the salivary gland library information led to identification of a vaccine candidate against *Leishmania major* (90) based on sand fly salivary allergens. Although we can identify with some certainty the possible role of some of the proteins synthesized with the obtained DNA information, the majority of the sequences are either of an unknown or unexpected nature.

UNEXPECTED SEQUENCES OR CONFLICTING STRATEGIES IN THE BLOODSUCKING ARENA

From their salivary gland cDNA library information, we have identified several unexpected activities in the blood-feeding strategy of arthropods that expanded our view of the salivary complexity of these animals. For example, in the sand fly *Lutzomyia longipalpis* we found, in addition to the ubiquitous apyrase activity that hydrolyzes ATP and ADP to AMP (14), a secreted 5′ nucleotidase hydrolyzing AMP to adenosine (69) and an adenosine deaminase that further hydrolyzes adenosine to inosine (13). While the 5′ nucleotidase may play a role in transforming AMP to the vasodilatory and antiplatelet compound adenosine, the primary vasodilator of Old World *Phlebotomus* sand flies (66), the conversion of adenosine to inosine does not fit with the salivary antihemostatic paradigm because inosine lacks vasodilatory and antiplatelet activities. We postulated that *L. longipalpis* might have evolved to produce these two salivary enzymes for two reasons. First, adenosine is a potent inducer of mast cell degranulation (86); second, because *Lutzomyia* has the potent peptidic vasodilator maxadilan, which does not exist in *Phlebotomus*, to produce vasodilatation, it has no need for the antihemostatic action of adenosine. If preventing mast cell degranulation by destroying adenosine is important for *Lutzomyia*, the unanswered question arises as to how *Phlebotomus* deals with mast cells, inasmuch as they secrete copious amounts of adenosine. It thus appears that preventing mast cell degranulation by destroying adenosine is important for *Lutzomyia*; like *Lutzomyia*, the mosquito *Ae. aegypti* appears to have the same dislike for adenosine. From mosquito salivary cDNA sequences, we found not only adenosine deaminase (65) but also purine nucleosidase sequences (96), which code for enzymes hydrolyzing inosine to hypoxanthine and ribose. The enzyme was indeed found in mosquito saliva, where it is one of the richest natural sources (78a). Finding this enzyme in any metazoan animal is unusual, as purine hydrolases of the enzyme class were thought to be exclusive of unicellular organisms and plants, not of animals. Perhaps *Aedes* destroys inosine because it still induces mast cell degranulation, albeit 10 times less potently than adenosine (86). Anopheline mosquitoes, however, do not have these adenosine/inosine-catabolizing enzymes, and how they deal with mast cells, if they do, remains to be discovered. Other enzymes found in both cDNA sequences (14) and insect saliva include hyaluronidase in sand flies and black flies (64), an enzyme that might help to spread the salivary pharmacologic agents through the skin matrix, and metalloprotease in ticks (I.M.B. Francischetti, T.N. Mather & J.M.C. Ribeiro, manuscript submitted),

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which may disrupt tissue repair. These enzymatic activity discoveries increase our understanding of the complex salivary potions of bloodsucking arthropods in general as well as show us the love/hate relationship of bloodsucking Diptera with adenosine, which raises the question of whether saliva evolution was influenced by host mast cells.

Many cDNA sequences coding for nonenzymatic peptides were also identified, together with a good presumption of their possible action. For example, the tick *I. scapularis* has a variety of specific protease inhibitors that have been confirmed by biochemical activity [described in (29, 94a)]. The variety of antiprotease activities in ticks points to the diverse cocktail they produce against host enzymes of the clotting cascade and inflammation.

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UNKNOWN SEQUENCES, OR THE PROBLEM OF ORPHAN MOLECULES

About 40% of the cDNA clusters we find from salivary gland libraries are of unknown function. In many cases, these sequences have a clear signal peptide indicating secretion, and their amino terminal or internal sequence, can be located by Edman degradation of protein bands transferred to PVDF membranes from saliva or salivary gland homogenates submitted to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Among the several classes of these unknown novel proteins, the D7 family exists in several bloodsucking dipteran species (93). The D7 family belongs to the odorant-binding superfamily but constitutes a distinct subset found in bloodsucking mosquitoes and sand flies. They are among the most abundant salivary proteins in these insects, have known allergenic properties (83), and may function by binding to host hemostatic/inflammatory agonists or serve to deliver some low-molecular-weight pharmacologic compound from the insect salivary gland to the vertebrate skin. After writing this review, a D7 protein from *Anopheles stephensi* was reported to inhibit activation of factor XII and prekallikrein (35a), thus having an effect in preventing bradykinin formation and activation of the intrinsic clotting pathway. Another ubiquitous and abundantly expressed family of salivary proteins in bloodsucking Diptera [from mosquitoes (96), sand flies (14) and tsetse (49)] is the antigen-5 family, a group of extracellular proteins found in wasp venom, seminal fluid, and plant defense proteins (81). Their function remains unknown. Sand fly (14) and mosquito salivary glands (96) also possess members of the *Drosophila* yellow protein family, which may have a function in oxidation of norepinephrine or DOPA agonists (37). Many putative peptide sequences (from 1 kDa to 15 kDa) are found in most bloodsucking arthropods studied thus far—often over 6–10 different peptides per species studied. Their biologic role is not evident and identifying their function remains a challenge. Because expression of large amounts of correctly folded proteins will be necessary for the many bioassays needed to identify their function, a network of specialized laboratories would be advantageous. Periodic comparison of such sequences to public databases will also help both to identify sequence similarities that may indicate function.

Obtaining crystal structure information of such proteins may also help elucidate their function.

POSSIBLE APPLICATIONS

It is clear that saliva of bloodsucking arthropods represents a vast range of novel molecules affecting hemostasis, inflammation, and immunity. With over 15,000 species in ~500 genera (73), we have barely scratched the surface of such biochemical and pharmacologic wealth. In addition, because the saliva of such arthropods affects the immune response of the host, it may become a novel vaccine target against various vector-borne diseases, as recently described for leishmaniasis (57, 90). The advanced salivary evolution of these bloodsucking insects may one day be the basis for alleviating not only the diseases they transmit but also other diseases of vascular origin. It is also appealing that these insects could be transformed with transposable elements (77) to synthesize and deliver vaccines—any vaccine—to their hosts.

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