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Role of Saliva in Tick/Host Interactions

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ABSTRACT

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Although several hosts mount efficient anti-tick immunity, natural tick/host associations are characterized by inefficient or non-existent anti-tick immunity. The absence of efficient anti-tick immunity in natural hosts could result from either host immune incompetence or the ectoparasite's successful evasion of the host's immune response. In this review I discuss data supporting the immune-evasion hypothesis and discuss its consequences to tick/host interactions.

INTRODUCTION

The classical paper authored by William Trager in 1939 indicated that guinea pigs were able to mount a very efficient immune response to the tick, *Dermacentor variabilis*. This immune response was characterized by a local reaction at the feeding site that prevented the tick from obtaining the blood meal. In addition, attachment of the tick to an immune host enhanced grooming, further reducing the ectoparasite's survival. Thus, a previously exposed quinea pig would allow only 3–5% of added larvae to feed, as opposed to more than 50% when naive animals served as hosts. A different result was obtained when the white-footed mouse *Peromyscus leucopus* was the host for *D. variablilis*. A single exposure would never confer anti-tick immunity and, after multiple exposures, only a partial immunity developed. Trager concluded that unnatural *D. variablilis* hosts, such as guinea pigs, were able to develop efficient anti-*Dermacentor* immunity, but natural hosts, such as the white-footed mouse, were not.

The immunological basis of tick resistance, studied mostly in guinea pigs, has been the subject of much research. Trager (1939) indicated that partial

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immunity could be transferred with serum, pointing to the mediation of antibodies in the rejection response; additional serum factors, such as the alternative-complement pathway, were implicated (Wikel and Allen, 1977). Cellular immunity also plays a role in tick rejection reactions; in particular, the role of basophils was well characterized and, indeed, tick/immune-host interactions became and experimental model for the study of the basophil-delayed hypersensitivity (Allen, 1973; Askenase, 1977; Brown et al., 1982). However, the lack, or weakness, of anti-tick immunity in natural tick/host associations remained poorly understood.

In theory, the lack of anti-tick immunity could be due to the host's inability to mount an effective immune response (host immune incompetence), or to the ectoparasite's ability to evade its host's immunological reaction (parasite immune evasion). The host immune incompetence theory has been assumed, without proof, by many investigators in their pursuit of effective anti-tick vaccines that could be useful in scenarios of natural tick/host associations (Wikel and Allen, 1982). The purpose of the present review is to give support to the hypothesis of parasite immune evasion in the explanation of stable tick/host associations, to outline a basic protocol for its test in different tick/host associations, and to speculate on its consequence in predicting the outcome of any tick/host interaction.

HOW UNNATURAL HOSTS REJECT TICKS

Edema is the most conspicuous histological observation at the tick feeding sites on resistant hosts (Tatchell and Moorhouse, 1968; Wikel and Allen, 1982). in some hosts, a basophil infiltrate exists which, through its released mediators, may increase vascular permeability at the feeding site and contribute to enhanced edema formation. Edema may be the significant component in tick rejection reactions. The availability of a nutritious meal is reduced because only a protein-poor serum transudate is available to the tick at edematous sites. Many ticks which survive this meal either do not molt or molt to abnormally small nymphs or adults (Wikel and Allen, 1982).

Many pharmacological mediators are efficient edema promoters. Among them are the vasoactive amines histamine and serotonin, the leukotrienes, particularly C4 and D4, and peptides such as bradykinin and the anaphylatoxins. Adenosine triphosphate, released by injured cells, also promotes edema. Prostaglandins, particularly of the E series, induce vasodilation and may potentiate the edema induced by the above agonists by increasing the hydrostatic perfusion pressure at the capillary bed (Williams and Peck, 1977; Bach, 1982; Ribeiro, 1987a).

Edema-promoting mediators may either be released from cells or result from acellular reactions. Basophils, mast cells and platelets have stored in their

granules abundant amounts of histamine or serotonin that can be released on contact with antigens, provided that a suitable antibody exists on their plasma membranes. Prostaglandins and leukotrienes are synthesized and released by activated leukocytes in varying amounts and proportions, depending on the cellular type. The most important cell-independent edema-promoting substances are bradykinin and the anaphylatoxins. Bradykinin is produced after activation of prekalikrein to kalikrein which may occur by triggering the blood-clotting cascade through the intrinsic pathway. Kalikrein, a serine protease, produces bradykinin from kininogen, a plasma globulin. Anaphylatoxins, cleavage peptides of the complement fractions C3 and C5, also result from cell-free proteolytic events triggered either by antigen/antibody complexes or suitable activating surfaces, such as agarose or zymosan (Bach, 1982).

Histamine and the alternative pathway of complement have been implicated in tick-rejection reactions. In both guinea pigs and in rabbits, resistance was partially abolished when histamine antagonists were given to previously tick-exposed hosts (Brossard, 1982; Wikel, 1982). Mice deficient in mast cells do not fully express anti-tick immunity, indicating a possible participation of histamine in this model (Matsuda et al., 1985, 1987). Guinea pigs lacking a complement response do not fully express their rejection reactions, indicating the participation of complement reactions, or their products, in tick-rejection reactions. Other mediators, perhaps leukotrienes, were implicated in anti-tick immunity but their nature is still obscure (Brown and Askenase, 1985).

In conclusion, hosts reject ticks by confronting the tick at the feeding site with a number of different mediators that may appear from cells already present at that site (such as mast cells), by cells chemotactically recruited to that site (such as basophils), and by cell-free reactions. The resulting reactions will both reduce the availability of blood for the tick and possibly produce toxic anti-tick products.

WHY NATURAL HOSTS DO NOT REJECT TICKS

Trager (1939) demonstrated that the white-footed mouse did not mount an efficient immunity to the immature instars of the tick *D. variabilis*, which naturally parasitises this host. In New England, the most common tick on *P. leucopus* is *Ixodes dammini, D. variabilis* immatures being mostly common in the vole *Microttus pennsylvanicus* (Spielman et al., 1985). Even after three exposure of 100 larvae of *I. dammini* to *P. leucopus*, no rejection was noticeable, while in control guinea pigs the rejection rate was high, with less than 5% successfully feeding (P. Davidar, M. Wilson and J.M.C. Ribeiro, unpublished data, 1989).

Saliva of adult *I. dammini* contain various pharmacologically active compounds that prevent platelet aggregation (Ribeiro et al., 1985), neutrophil aggregation (J.M.C. Ribeiro, unpublished data, 1988), and T-cell activation (Ri-

beiro et al., 1985). ATP and ADP are very quickly converted to AMP through a salivary ATP-diphosphohydrolase (apyrase). Bradykinin and anaphylatoxin are inactivated, possibly by a basic carboxypeptidase (Ribeiro and Spielman, 1986). Complement C3 fixation to activating surfaces is inhibited (Ribeiro, 1987b). Prostaglandin E_2 (Ribeiro et al., 1985) and prostacyclin (J.M.C. Ribeiro, G. Makoul and D. Robinson, unpublished data, 1988) are secreted in pharmacologically active amounts; these substances may prevent the release of vasoactive amines and inflammatory eicosanoids from leukocytes (Bach, 1982) and at the same time produce vasodilation bringing more blood to the tick's mouthparts. It thus appears that the complexity of the hosts's pharmacological mediators of inflammation and edema have a parallel in the complexity of the pharmacological mediators in the tick saliva.

However, absent from *I. dammini* saliva is a histamine antagonist. Following the same protocols that demonstrated anti-histaminic activity in saliva of the triatomine bug *Rhodnius prolixus* (Ribeiro, 1982), no anti-histaminic activity was found in *I. dammini* saliva. Incubation of histamine with saliva did not reveal histaminase activity (J.M.C. Ribeiro, unpublished data, 1987). From the pharmacological profile of *I. dammini* saliva, it can be concluded that this tick is well adapted to avoid those edema mediators that are mainly produced by cell free reactions, such as anaphylatoxins, and kinins, but unable to evade histamine-originated edema.

Vertebrates differ in the relative importance of each inflammatory agonist, and even in the relative amounts of effector cells involved in inflammatory processes. Guinea pigs, for example, have their anaphylatic reactions mediated by histamine. Experimental asthma in such animals can be prevented with histamine antagonists, while in humans, leukotrienes seem to have a predominant role, anti-histaminics being of limited or no value. Histamine-rich basophils are very abundant in guinea pigs, in which the cells concentrate at tick feeding sites, but this cell type is virtually absent in the laboratory mouse (Askenase, 1977). This may be the reason why guinea pigs mount such strong anti-tick immunity and mice display only a partial anti-tick immune response which is mast-cell-dependent (Matsuda et al., 1985, 1987).

From the above discussion, it is concluded that the key to the successful parasitism of *P. leucopus* by *I. dammini* is the tick's ability to evade the host repertoire of immunopharmacological agonists by counter-producing a number of salivary antagonists. If the edema produced by a host relies on mediators which the tick can counteract, successful repeated parasitism may exist. Otherwise, expression of anti-tick immunity may become apparent.

CONCLUSION

At the risk of oversimplification, it can be proposed that natural tick/host associations are maintained by the tick's salivary anti-edema components. If

this statement is confirmed, it would be possible, knowing a particular host's edema-promoting agonists and a particular tick's salivary pharmacological activities, to predict whether that tick/host combination would lead to successful parasitism, or which characteristics a host should possess to prevent successful parasitism.

Confirmation of this hypothesis awaits more systematic investigation on different tick/host associations, both on the nature of the host's physiological agonists of edema and on the tick's putative anti-edema activities. Regarding cattle/tick associations, it is known that *Boophilus microplus*-resistant cattle (*Bos indicus*) have a greater skin histamine content than susceptible cattle (*B. taurus*) (Willadsen et al., 1979). Does the cattle tick, like *I. dammini*, have no salivary anti-histaminic activity? Other ticks, like *Rhipicephalus sanguineus* have salivary anti-histaminic activity (Chinery and Ayitey-Smith, 1977) that could allow this tick to feed on more-histaminergic hosts. Does *Rhipicephalus* counteract efficiently the anaphylatoxins or bradykinin? Do histaminergic animals like guinea pigs mount a good tick immunity to *Rhipicephalus*? Answers to these questions may clarify some aspects of tick parasitism and indicate phenotypic markers for breeding tick-resistant animals.

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