Tick immunobiology

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SUMMARY

Ticks are of vast medical and veterinary public health importance due to direct damage caused by feeding and their roles in transmitting well known and emerging infectious agents. Ticks and tick-borne pathogens stimulate the immune system of the host. Those immune interactions are of importance in tick biology, pathogen transmission and control of ticks and tick-borne diseases. Both innate and specific acquired immune defenses are involved in the responses of vertebrate hosts to infestation. Ticks have evolved countermeasures to circumvent host immune defenses. This review addresses the immunobiology of the tick–host interface from the perspectives of the pharmacology of tick saliva; relationship of tick saliva to pathogen transmission; host immune responses to infestation; tick modulation of host immune defences; and genomic/proteomic strategies for studying tick salivary gland molecules.

Key words: Ticks, immunobiology, immunomodulation, saliva, genomics, proteomics.

INTRODUCTION

Ticks are of vast importance due to their ability to transmit an impressive variety of infectious agents and to cause direct injury by piercing host skin (Sonenshine, 1991, 1993). The resurgence of well known vector-borne diseases and emergence of new infectious agents are significant global public health concerns (Gratz, 1999). Emerging arthropod-transmitted infectious agents of wildlife are potential zoonotic disease threats (Daszak, Cunningham & Hyatt, 2000). The worldwide picture of ixodid tick-transmitted bacterial diseases is an example of this dynamic situation with 15 previously unrecognized bacterial pathogens described since 1982 (Parola & Raoult, 2001; see also chapter by Telford & Goethert in this Supplement). Reviews focus on tick-borne diseases of medical importance (Shapiro, 1998; Walker, 1998; Childs & Paddock, 2003). An issue of Medical Clinics of North America, edited by Edlow (2002), provides a multi-author, multi-chapter, comprehensive treatment of tick-borne diseases. Control of arthropod-borne pathogens is complicated by a lack of vaccines (Walker, 1998), drug resistance (Molyneux, 1998) and the development of tick resistance to acaricides (Mitchell, 1996; see also chapter by George, Pound & Davey in this Supplement). In addition to traditional approaches, novel methods are needed for suppression of tick populations and control of tick-borne infectious agents. Increasingly, attention has focused on vaccine-based control of ticks (Wikel, Alarcon-Chaidez & Mueller-Doblies, 2003). Characterizing the immunobiology of the tick–host interface is essential for understanding tick feeding, pathogen transmission and development of anti-tick vaccines.

Dramatic advances in studying the immunobiology of tick–host interactions have been achieved during the past three decades. Innate and specific acquired immune responses elicited by tick feeding depend on both the species of tick and of host. Variations in host genetic composition are well recognized factors in determining immune responses to infestation (de Castro & Newson, 1993). Importantly, interspecies and intraspecies variations in the composition of tick saliva must be considered. Differences could arise in relatively isolated populations that occur over the geographic range of a tick species. Likewise, variations are expected to occur in the salivary secretions of individual ticks within a population (Wang, Kaufman & Nuttall, 1999). In addition to assessing the mechanisms of innate and specific acquired resistance to tick feeding, a considerable body of research has focused on tick modulation or deviation of host immune responses (Wikel, 1996, 1999; Gillespie, Mbow & Titus, 2000; Schoeler & Wikel, 2001). Salivary gland molecules responsible for these activities are beginning to be identified. Modulation of host defenses is increasingly linked to pathogen transmission (Schoeler & Wikel, 2001; see also chapter by Nuttall & Labuda in this Supplement). The pace of discovery will increase rapidly with application of the the tools of genomics, functional genomics and proteomics to the dissection of tick host-pathogen interactions (Valenzuela, 2002*a*).

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PHARMACOLOGY OF TICK SALIVA

In addition to immune defenses, haematophagous arthropods must overcome host blood coagulation, platelet aggregation and pain/itch responses in order to obtain a blood meal successfully. Different solutions to these problems have evolved with selected elements of these host defence pathways more commonly targeted than others by these sophisticated pharmacologists (Ribeiro, 1995*a*). An important point is that ticks have evolved redundant systems for counteracting host defences (Ribeiro, 1995*a*, *b*). Reviews of the pharmacology of blood-feeding arthropod saliva are instructive and provide the platform for recent studies (Ribeiro, 1989; Titus & Ribeiro, 1990; Champagne, 1994; Champagne & Valenzuela, 1996; see also chapter by Valenzuela in this Supplement). In this section we will focus on saliva molecules that impact host immune defences.

Advances in tick immunobiology have resulted from identification, and in many cases cloning and expression, of tick salivary gland proteins that interact with elements of host immune systems. A secreted calreticulin of *A. americanum* was cloned from tick salivary glands (Jaworski *et al*. 1995). This calreticulin lacks an endoplasmic reticulum retention signal and it is secreted in the saliva of four-day fed females of both *A. americanum* and *D. variabilis*. Calreticulin of *B. microplus* was recently cloned and partially characterized (Ferreira *et al*. 2002). The predicted molecular weight of this molecule was 47·7 kDa, based on translated protein sequence from the nucleic acid sequence of the open reading frame. The *B. microplus* calreticulin is expressed in all tissues and all developmental stages (Ferreira *et al*. 2002). *A. americanum* calreticulin induces an antibody response in humans exposed to tick bite (Sanders *et al*. 1998). In contrast, cattle given eight experimental infestations with *B. microplus* did not develop antibodies to calreticulin, and immunization of a bovine with recombinant calreticulin did not induce an IgG response (Ferreira *et al*. 2002). Tick calreticulins show closer phylogenetic relationships to mammalian than arthropod calreticulins (Ferreira *et al*. 2002), which may be linked to their possible biological functions. Calreticulins are conserved calcium-binding proteins with a diversity of biological functions from molecular chaperones, extracellular lectins, modification of gene expression by binding to nuclear hormone receptors, antihaemostasis and binding of the globular head of complement component C1q (Benedict *et al*. 1993; Coppolino & Dedhar, 1998; Kovacs *et al*. 1998). The roles of these molecules in tick–host interactions remain to be determined.

Immunoglobulin G-binding proteins are present in salivary gland extracts (SGEs) of *Amblyomma variegatum*, *Ixodes hexagonus*, and *Rhipicephalus appendiculatus* (Wang & Nuttall, 1995). Five similar immunoglobulin G-binding bands were observed by SDS–PAGE for unfed male and six day fed female *R. appendiculatus*. The molecular weights ranged from 21 to 54 kDa, and six day fed *R. appendiculatus* males possess a different molecular weight pattern of reactive proteins. Likewise, the immunoglobulin G-binding proteins differ in molecular weight for these three tick species (Wang & Nuttall, 1995). Immunoglobulin-binding proteins could be important in eliminating from the tick those antibody molecules which cross the tick gut. Immunoglobulins are a major component of host serum, and they can cross the tick gut and react with tissues in the haemocoel (Allen & Humphreys, 1979).

Interleukin-2 is a cytokine that possesses many biological activities important for immune response development and expression (Janeway *et al*. 2001). Saliva of *I. scapularis* contains an interleukin-2 (IL-2) binding protein that complexes with the cytokine in the fluid phase and appears to not require direct interaction with the IL-2 producing T-cell (Gillespie *et al*. 2001). Both human and mouse IL-2 are bound with similar affinities. Interleukin-2 binding protein can affect the activity of those cells that express IL-2 receptors: activated T-cells, B-cells, NK cells, cytotoxic T-cells, monocytes and macrophages (Gillespie *et al*. 2001). Targeting host cytokines is an effective strategy for the tick to regulate potentially damaging host responses.

Histamine has been linked to expression of acquired resistance to tick feeding (Wikel, 1982). Histamine and serotonin reduced salivation and feeding by *D. andersoni* (Paine, Kemp & Allen, 1983). Mast cells situated in a perivascular location would be a source of histamine to which the feeding tick would be exposed. *R. appendiculatus* salivary glands contain histamine-binding proteins (Paesen *et al*. 1999). Two histamine-binding proteins are present in females and one in males. Female proteins are expressed early in feeding with a peak at 48 hours, and they are not present in males, nymphs or larvae. The male protein is expressed throughout feeding and it is present in nymphs and larvae. Histamine-binding proteins are highly specific; they contain two histamine-binding pockets with different affinities and binding is different from mammalian H1 and H2 receptors (Paesen *et al*. 1999). Very likely, histamine-binding proteins will be found in the majority, if not all, tick species.

Complement is a series of serum proteins which are activated through the classical, alternative and mannose-binding pathways and function as important mediators of inflammation, chemotaxis, opsonisation and microbe clearance (Fearon, 1998; Mastellos & Lambris, 2002). The alternative pathway of complement activation is involved in acquired resistance to tick feeding (Wikel, 1979). An anticomplement protein has been purified from *I. scapularis* saliva, cloned and expressed in mammalian

cells (Valenzuela *et al*. 2000). This protein has a molecular mass of 18·5 kDa and appears to inhibit C3b binding and accelerates uncoupling of factor Bb from the alternative pathway C3 convertase. Both tick saliva and the recombinant protein act in a similar manner.

A 36 kDa protein identified in the saliva of *D. andersoni* inhibits mitogen driven T-lymphocyte proliferation (Bergman *et al*. 2000). Antibodies reactive with this protein bound polypeptides of 33 kDa and 101 kDa of a soluble protein extract of *D. variabilis* salivary glands (Bergman *et al*. 2000). The pleotropic lymphocyte/macrophage pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF), is expressed in salivary gland and midgut of *A. americanum* (Jaworski *et al*. 2001). Tick MIF is present in unfed and three-day fed female salivary glands. A number of biological activities have been attributed to MIF, including oxidoreductase activity, neurohumoral mediator and inhibiton of NK cell mediated lysis (Petrovsky & Bucala, 2000; Lue *et al*. 2002).

Antibodies derived from guinea pigs expressing acquired resistance to *I. scapularis* nymphs have been used to identify immunodominant proteins by screening a cDNA library prepared from engorged nymphs (Das *et al*. 2000). *Salp16* is a gene induced during feeding, which encodes a putative 16·4 kDa protein present in engorged, but not unfed, nymphs and adults (Das *et al*. 2000). Salp16 represents approximately 0·2% of the total protein in saliva, and immunization with the recombinant form of this protein does not induce a protective response against tick infestation. Salp 25D is a homologue of glutathione peroxidase, and the recombinant form of this protein catalyzes the reduction of H_2O_2 in the presence of reduced glutathione and glutathione reductase (Das *et al*. 2001). Salp 25D has partial homology to the histamine-binding proteins of *R. appendiculatus* (Das *et al*. 2001). Salp15 is an inhibitor of CD4+ T-lymphocyte activation by acting through repression of calcium-dependent signals initiated through engagement of the T-cell receptor (Anguita *et al*. 2002). Salp15 binds to T-cells, inhibits IL-2 and CD25 expression, reduces delayed type hypersensitivity responses, and does not affect T-cell independent B-cell responses.

Salivary prostaglandin E_2 and I_2 are vasodilators (Ribeiro, 1995*a*) and inhibitors of platelet aggregation (Champagne & Valenzuela, 1996). Tick saliva often contains high concentrations of prostaglandins (Bowman, Dillwith & Sauer, 1996). Prostaglandin E_2 (PGE_2) occurs in the salivary glands of *Boophilus microplus* (Dickinson *et al*. 1976; Higgs *et al*. 1976), *I. scapularis* (Ribeiro *et al*. 1985) and *A. americanum* (Ribeiro *et al*. 1992). Prostaglandins have divergent roles in regulation of immune responses (Harris *et al*. 2002). Their importance in modulation of host immune rsponses to ticks is unclear and definitive studies are needed. Prostaglandin E_2 does enhance production of Th2 cytokines and down regulate Th1 cytokines and inhibit T-lymphocyte proliferation (Harris *et al*. 2002). Similar findings have been reported for T-cells from tick exposed hosts or T-cells exposed to tick salivary gland proteins (see below). Urioste *et al*. (1994) observed that *I. scapularis* inhibition of T-cell proliferation is not prostaglandin dependent.

TICK SALIVA AND POTENTIATION OF PATHOGEN TRANSMISSION

Saliva of blood-feeding insects and ticks potentiate infection with a variety of vector-borne disease-causing agents. The precise mechanisms of heightened infectivity are not clearly defined; however, modulation of host immune defenses is speculated to be a key element (Schoeler & Wikel, 2001). The seminal observation was made by Titus & Ribeiro (1988) that *Lutzomyia longipalpis* salivary gland homogenate heightened the numbers of *Leishmania major* in cutaneous lesions up to 5000 times and the size of lesions up to ten times when salivary gland homogenate and promastigotes were injected together into the footpads of mice. Infection with Cache Valley virus was enhanced when virus was inoculated into sites where uninfected *Aedes aegypti*, *A. triseriatus* or *Culex pipiens* mosquitoes had fed within the previous four hours (Edwards, Higgs & Beaty, 1998). In that study, mosquito thorax extract, containing mosquito salivary glands, did not potentiate virus infection.

Tick salivary gland molecules also potentiate infection with several infectious agents (see chapter by Nuttall & Labuda in this Supplement). Tick feeding within 12 cm of the subcutaneous inoculation site of Thogoto virus with SGE of uninfected six-day fed female *R. appendiculatus* on guinea pigs increased the number of uninfected co-feeding ticks that became virus infected by ten fold, suggesting that virus transmission was enhanced by tick saliva (Jones, Hodgson & Nuttall, 1989). A similar phenomenon was observed for guinea pigs administered tick-borne encephalitis virus in combination with extracts of salivary glands derived from partially engorged female *I. ricinus*, *D. reticulatus* or *R. appendiculatus* (Labuda *et al*. 1993). SGEs of unfed or four-day fed *R. appendiculatus* enhanced susceptibility of *Bos indicus* lymphocytes to infection with *Theileria parva* (Shaw, Tilney & McKeever, 1993). SGEs of *D. reticulatus* and *R. appendiculatus* dramatically increased vesicular stomatitis virus (VSV) growth in mouse L cells (Hajnická *et al*. 1998). Tick SGE increased VSV viral nucleocapsid protein and phosphoprotein in a dose-dependent fashion (Kocakova *et al*. 1999). *Dermacentor reticulatus* SGE inhibits the effects of interferon α/β of mouse L cell origin on the replication of VSV in L cells (Hajnická *et al*. 2000).

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Co-feeding transmission of Thogoto virus occurs when *R. appendiculatus* larvae feed on A2G mice, which are resistant to Thogoto virus, that are concurrently being infested with virus-infected nymphs, suggesting that tick saliva components overcome host anti-virus defences (Dessens & Nuttall, 1998).

Since tick saliva potentiates pathogen infectivity, development of immunity to molecules in tick saliva might reduce transmission of infectious agents, and that appears to occur. Rabbits exposed to feeding by uninfected *D. andersoni* developed resistance to subsequent transmission of fully virulent *Francisella tularensis* by infected adult *D. andersoni* (Bell, Stewart & Wikel, 1979). Thogoto virus-infected *R. appendiculatus* adults fed on tick-resistant guinea pigs infected significantly fewer co-feeding nymphs than when a similar experiment was performed with guinea pigs that were not tick resistant (Jones & Nuttall, 1990). A natural host, *Clethrionomys glareolus*, develops resistance to *I. ricinus* feeding, which was thought possibly to interfere with *Borrelia burgdorferi sensu lato* transmission (Dizij *et al*. 1994). Although acquired resistance to tick feeding did not occur, mice given four infestations with pathgoen-free *I. scapularis* nymphs were resistant to *B. burgdorferi* transmission by a subsequent infestation with infected nymphs (Wikel *et al*. 1997). Repeated infestations of guinea pigs with uninfected *I. scapularis* nymphs acquired resistance to both infestation and subsequent transmission of *B. burgdorferi* by infected nymphs (Nazario *et al*. 1998).

These observations support the hypothesis that a vaccine that targets and neutralizes molecules essential for transmission of tick-borne infectious agents will provide protection against infection. The predominant focus for this approach is the immunomodulatory proteins in tick saliva (Schoeler & Wikel, 2001). An alternative approach is to identify saliva proteins that stimulate an intense host response at the bite site, which will indirectly kill the introduced infectious agent (Valenzuela *et al*. 2001; see also chapter by Valenzuela in this Supplement). If proven to be effective, these strategies could circumvent the need to develop vaccines against each and every tick-transmitted pathogen.

IMMUNE RESPONSE TO TICK INFESTATION

After the chelicerae and hypostome of the tick penetrate host skin a local inflammatory response develops in which host neutrophils participate. The chemokine IL-8 controls the movement and activity of neutrophils. Significantly, SGE from several tick species (*D. reticulatus*, *A. variegatum*, *R. appendiculatus*, *Haemaphysalis inermis* and *I. ricinus*) inhibits *in vitro* binding of IL-8 to its receptors on human granulocytes, which have been enriched for neutrophils (Hajnická *et al*. 2001). In this manner, tick saliva could control, in part, infiltration and activation of neutrophils at the bite site. Ticks feed on a pool formed by destruction of tissue and blood vessels beneath the tip of the mouthparts. Tick salivary glands, which undergo dramatic morphological and functional changes during the course of engorgement, produce factors essential for normal feeding (Kaufman, 1989) and pathogen transmission (Brossard & Wikel, 1997).

Some tick–host relationships are characterized by the acquisition of resistance to tick feeding which develops as a result of repeated infestations (Willadsen, 1980; Wikel, 1996). Acquired resistance to ticks is expressed as reduced engorgement weight, increased duration of feeding, decreased numbers of ova, reduced viability of ova, blocked moulting and death of engorging ticks (Bowessidjaou, Brossard & Aeschlimann, 1977; Wikel, 1996). *Ixodes ricinus* females feeding on immune rabbits digest haemoglobin with difficulty (Papatheodorou & Brossard, 1987). As early as 1939, Trager observed that guinea pigs become strongly immune to *D. variabilis* larvae (Trager, 1939). Since that time, immunity to ticks has been most extensively studied using laboratory animals (guinea pigs, rabbits or mice) and bovines (Willadsen, 1980; Brossard & Wikel, 1997; Willadsen & Jongejan, 1999). Adaptive anti-tick immunity in laboratory animals is often more intense than that observed for natural hosts (Ribeiro, 1989). Laboratory mice infested with *I. ricinus* (Mbow *et al*. 1994), *I. scapularis*, *I. pacificus* (Schoeler, Manweiler & Wikel, 2000) or *R. sanguineus* (Ferreira & Silva, 1999) immature stages are exceptions as they do not acquire resistance. In contrast, reduction in numbers of engorged ticks and in weights of fed larvae are observed on BALB/c mice repeatedly infested with *D. variabilis* (denHollander & Allen, 1985). However, some wild rodents (*Clethrionomys glareolus* for example) do acquire immunologically-based resistance to ticks (Dizij & Kurtenbach, 1995).

It has been known for a long time that some breeds of cattle develop immunity after repeated infestations with ticks (reviewed in Willadsen, 1980, 1987). The *B. microplus*-bovine host–parasite relationship is the most extensively studied from an immunological perspective. Acquired resistance or susceptibility of bovines were described for infestations with *Hyalomma* spp. (Sahibi *et al*. 1997), *H. anatolicum anatolicum*, *Rhipicephalus evertsi* (Latif, 1984) and *R. appendiculatus* (Latif *et al*. 1991).

Tick feeding induces a complex array of host immune responses involving antigen presenting cells (APC), T-cells, B-cells, antibodies, cytokines, complement, basophils, mast cells, eosinophils and a number of bioactive molecules (reviewed in Wikel, 1996; Brossard & Wikel, 1997). These complex interactions can be viewed as a balance between host defences raised against the parasite and tick evasion strategies, facilitating feeding and transmission of pathogens. Circulating IgG antibodies to saliva

antigens, which are induced by tick feeding, are detected in different host animals (Brossard, 1976; Brossard, Rutti & Haug, 1991; Wikel, 1996). By passively transferring immune serum to naive laboratory animals, it was shown that humoral factors are involved in the acquisition of immunity against ticks (Brossard & Girardin, 1979). Immunity against *B. microplus*, even though weakened, was also transmitted passively to cattle (Roberts & Kerr, 1976). Antibodies are not the only effector elements of the immune system contributing to acquired resistance.

The role of lymphocytes in expression of acquired resistance to *D. andersoni* larvae was proven by adoptively transferring lymph node cells from resistant guinea pigs into naive animals (Wikel & Allen, 1976). Involvement of T-cells was also established by applying the immunosuppressor cyclosporin A to rabbits infested with *I. ricinus* adults (Girardin & Brossard, 1989, 1990). T-cells are key elements in regulation and effector functions of the immune system, including antibody production and cell mediated immunity. Lymphocytes derived from infested laboratory animals (Wikel, Graham & Allen, 1978; Schorderet & Brossard, 1994; Ganapamo, Rutti & Brossard, 1997) and cattle (George, Osburn & Wikel, 1985) undergo *in vitro* blastogenesis when cultured in the presence of SGE. Reactivity is generally more intense with cells obtained during repeated exposures. In BALB/c mice repeatedly infested with nymphs of *I. ricinus*, lymphocytes from lymph nodes, which drain the tick attachment site, produce significant levels of TNF-α and granulocyte/monocyte colony stimulating factor (GM-CSF), when stimulated *in vitro* with ConA or anti-CD3 antibodies (Ganapamo, Rutti & Brossard, 1996*a*). GM-CSF induces the maturation of Langerhans cells (LCs) *in vitro* by maintaining their viability and inducing high expression of Ia molecules (Berthier *et al*. 2000). LC migration from the skin to the regional lymph nodes and their accumulation in these secondary lymphoid organs are controlled by TNF-α (Cumberbatch & Kimber, 1995). Langerhans cells in the skin trap antigens, as well as interacting with a variety of cell types through an array of cytokines (Salmon, Armstrong & Ansel, 1994). LCs are one of the first cells to be exposed to tick immunogens in the skin, from which they migrate to draining lymph nodes (Allen, Khalil & Wikel, 1979). In the paracortical area of the lymph nodes LCs transform into dendritic cells (DCs) and function there as APCs for T-cells (Nithiuthai & Allen, 1984*a*). Ultraviolet radiation treatment of guinea pig ears before primary infestation with *D. andersoni* larvae reduced the number of LCs and acquired resistance (Nithiuthai & Allen, 1984*b*). When UV is applied to resistant animals a marked diminution of resistance during reinfestation was observed. Short wavelength UVC was found to be more effective than mid-wavelength UVB in depleting LCs (Nithiuthai & Allen, 1984*b*). Functional LCs are necessary to initiate adaptive immunity and to stimulate the animals for a secondary immune response.

The importance of DCs to induce an anti-tick immune response was recently studied by Lorimier & Brossard (personal communication). Murine spleen DCs were purified and separated into different subsets: CD8α+ and CD8α− (CD4+ or CD4−). Each subset was pulsed *in vitro* with *I. ricinus* female saliva and injected into naive BALB/c mice. The whole population of DCs and CD8α− DCs triggered a Th2 immune response. In contrast, CD8α+ DCs polarized the response towards Th1. Whole spleen DCs pulsed with tick saliva were able to prime naive splenic cells *in vitro*. Using RT-PCR, elevated amounts of IL-4 mRNA were detected in proliferating cells, demonstrating establishment of a primary Th2 immune response *in vitro* (Mejri & Brossard, personal communication).

The complement system is also involved in development of immunity against ticks. Acquired resistance to *D. andersoni* larvae in guinea pigs was inhibited by dramatically lowering the levels of C3 with cobra venom factor (Wikel & Allen, 1977). C4-deficient guinea pigs acquired resistance showing that the alternate pathway of complement activation was important in the expression of this immunity (Wikel, 1979). C3 was deposited in the dermal-epithelial junction near the location of the tick bite (Allen *et al*. 1979). The SGEs of *I. ricinus*, *I. hexagonus* and *I. uriae* inhibit the alternative pathway of complement, indicating the importance of that component of innate immunity in the response to tick feeding (Lawrie, Randolph & Nuttall, 1999). Activation of the complement system in the vicinity of the bite site would result in generation of mediators of inflammation, chemotaxis, opsonins and could locally attract basophils and other cells linked to host resistance.

Cutaneous reactions at tick attachment sites on cattle and laboratory animals expressing acquired immunity contain infiltrates of basophils and eosinophils (Allen, 1973; Allen, Doube & Kemp, 1977; Brossard & Fivaz, 1982; Steeves & Allen, 1991). This type of reaction is termed cutaneous basophil hypersensitivity (CBH), which is a form of delayed type hypersensitivity mediated by Th1 cells (Askenase *et al*. 1978). Infiltration of basophils was more pronounced in *D. andersoni*-infested guinea pigs (Allen, 1973) than in *I. ricinus*-infested rabbits (Brossard & Fivaz, 1982). CBH developed also after the infestation of rabbits or cattle with *H. a. anatolicum* (Gill & Walker, 1985; Gill, 1986) and guinea pigs or cattle infested with *I. holocyclus* (Allen *et al*. 1977) or *A. americanum* (Brown & Askenase, 1981; Brown, Barker & Askenase, 1984). Saliva antigens complex with homocytotropic antibodies bound to Fc receptors on mast cells and basophils. Those cells are activated and release bioactive molecules, including

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Table 1. Overview of cells and molecules of the host immune system and tick countermeasures

Cell or molecule	Role in immune defence	Tick countermeasure	Possible significance
Complement (general)	Classical, alternative, mannose-binding pathways to generate, inflammatory mediators and opsonins, C3 pivotal molecule for all three pathways.	C3b deposition inhibited by *I. scapularis* saliva (Ribeiro, 1987).	Reduce inflammatory response and lysis of microbes.
Alternative complement pathway	Antibody independent, mediates inflammation and direct lysis of microbes.	Role in acquired resistance to *D. andersoni* (Wikel, 1979). Inhibited by SGEs of *I. ricinus*, *I. hexagonus*, *I. uriae* (Lawrie *et al.* 1999). Binding of C3B and uncoupling Bb by *I. scapularis* 18·5 kDa protein (Valenzuela *et al.* 2000).	Reduce inflammatory response to tick and tick-transmitted microbes.
Anaphylatoxin	Complement components C3a, C4a, C5a bind to cells and promote acute inflammation, neutrophil chemotaxis, mast cell activation.	*I. scapularis* saliva antagonist of anaphylatoxins (Ribeiro & Spielman, 1986).	Reduce cellular infiltrate and release of mast cell mediators (histamine, leukotrienes, cytokines).
NK cells	Lymphocytes (neither B nor T) that directly kill microbe infected cells and secrete IFN-γ.	SGE of *D. reticulatus* effector function of NK cells from healthy humans, affecting effector-target cell interaction (Kubes *et al.* 2002). Similar, but lesser activity in SGEs of *A. ariegatum* and *H. inermis* (Kubes *et al.* 2002).	Reduce killing of microbe infected cells and suppress IFN-γ, which activates macrophages and can polarize acquired immune response to Th1 profile.
Chemokines	Cytokines that stimulate leukocyte migration (chemotaxis) from blood into tissues. Numerous different chemokines identified, including IL-8.	Anti-IL-8 activity in SGEs of *A. variegatum*, *D. reticulatus*, *H. inermis*, *I. ricinus* and *R. appendiculatus* (Hajnická *et al.* 2001).	IL-8 is chemotactic for neutrophils, basophils and T-lymphocytes. All of these cells have roles in host immune responses to limit tick feeding. Blocking IL-8 confers a survival advantage for the tick.
Adhesion molecules	Cell surface molecules that interact with other cells or extracellular matrix to promote cell migration and activation (also in adaptive immunity).	Infestation of BALB/c mice with *D. andersoni* nymphs reduced lymphocyte expression of the lymphocyte integrins LFA-1 and VLA-4, which respectively bind to the adhesion molecules ICAM-1 & 2 and VCAM-1 on activated endothelium (Macaluso & Wikel, 2001).	Modulation of host leukocyte migration to tick bite site and reduced immune response to infestation.
Monocyte/Macrophage function	Blood monocytes become tissue macrophages, which are phagocytic and activated by IFN-γ. Activated macrophages kill engulfed microbes, present antigens to CD4 T-lymphocytes and secrete pro-inflammatory cytokines (TNF-α, IL-1β). Macrophage derived IL-12 promotes IFN-γ from NK and T-cells to activate macrophages. NO is involved in intracellular killing of microbes by macrophages.	Macrophage IL-1β and TNF-α production reduced by *D. andersoni* SGE (Ramachandra & Wikel, 1992). Saliva of *I. scapularis* suppressed macrophage NO production (Urioste *et al.* 1994). Saliva of *R. sanguineus* inhibits IFN-γ macrophage activation and reduced NO production (Ferreira & Silva, 1998). SGE of *I. ricinus* reduced production of NO (Kopecky & Kuthejlova, 1998). SGE of *I. ricinus* inhibits macrophage killing of *Borrelia*	Prevent inflammatory responses, reduce cytokines that could orchestrate anti-tick responses, reduce ability to clear microbes, and possibly alter antigen presentation.

		afzelii (Kuthejlova *et al*. 2001). SGE of *R. appendiculatus* inhibits *in vitro* transcription and secretion of IL-1α, TNF-α, IL-10 and production of NO by murine macrophage cell line (Gwakisa *et al*. 2001). Macrophage migration inhibition factor in salivary glands of *A. americanum* (Jaworski *et al*. 2001) has reported roles inhibiting NK cell mediated lysis and delayed type hypersensitivity responses.	
Histamine-binding proteins and serotonin-binding proteins	Histamine and serotonin are mediators of the itch response. Histamine increases vascular permeability and it is a mediator of inflammation. Concurrent blocking of type-1 and type-2 histamine receptors reduced acquired resistance to *D. andersoni* (Wikel, 1982). Histamine and serotonin reduce sucking and salivation of *D. andersoni* (Paine *et al*. 1983).	Histamine blocking by SGE of *R. sanguineus* (Chinery & Ayitey-Smith, 1977). Histamine-binding proteins in SGE of *R. appendiculatus* (Paesen *et al*. 1999). Lipocalin of *D. reticulatus* binds histamine and serotonin (Sangamnatdej *et al*. 2002).	Reduction of inflammation and itch response at bite site. Reduce direct impact of histamine and serotonin on tick feeding.
Lymphocyte cytokine modulation	Cytokines mediate differentiation/activation of lymphocytes and other cells and orchestrate innate and adaptive immune responses. IL-2: T-cell, NK cell and B-cell proliferation and antibody synthesis. IL-4: Th2 polarization/differentiation and proliferation, inhibits macrophage activation, and mast cell proliferation. IL-10 inhibitor of IL-12, alters expression of MHC class II and co-stimulatory molecules, inhibits activated dendritic cells/macrophages. IFN-γ activates macrophages and polarizes toward Th1 response.	Influence of ticks on host cytokines have been the focus of numerous studies. A general pattern that has emerged is down-regulation of Th1 responses and polarization toward Th2 cytokine profiles (see text and review by Schoeler & Wikel, 2001).	Modulation or deviation of T-lymphocyte cytokines can reduce immune responses to tick feeding and in turn facilitate transmission/establishment of microbes.
Lymphocyte proliferation	Proliferation of T and B-lymphocytes is essential for increased population and to perform effector functions. Numerous factors and receptor-ligand interactions influence lymphocyte proliferation. Cell mediated immune responses linked to acquired resistance.	Several investigators reported tick suppression of *in vitro* T-cell proliferation as a result of infestation or exposure of lymphocytes *in vitro* to saliva, salivary gland derived molecules, or recombinant salivary gland proteins (see text and review by Schoeler & Wikel, 2001).	Reduce immune responses to tick saliva proteins, which also reduce host immunity to tick-transmitted microbes.
Antibody responses	Antibodies carry out a variety of functions including neutralization, complement fixation (classical pathway), and facilitating leukocyte interaction with target cells (including microbes). Antibodies linked to expression of acquired resistance.	Tick infestation reduces antibody responses to heterologous antigens, but the basis for this suppression is unknown.	Reduce the likelihood of developing antibodies that could neutralize tick saliva proteins essential for successful blood feeding.

histamine, leukotrienes, prostaglandins and enzymes at the bite site which contribute to expression of acquired immunity (Brossard & Fivaz, 1982; Brossard, Monneron & Paptheodorou, 1982; Wikel, 1996). A greater number of degranulated cells was found during reinfestation (Brossard & Fivaz, 1982; Schleger *et al*. 1976). In cattle infested with *B. microplus*, release of histamine causes skin irritation which leads to increased host grooming. In this way, some ectoparasites are actively removed from the host (Koudstaal, Kemp & Kerr, 1978). A positive correlation was established between skin histamine concentration and the degree of resistance acquired by cattle against *B. microplus* (Willadsen, Wood & Riding, 1979). In guinea pigs infested with *D. andersoni*, histamine-rich basophils concentrated in the area around the attachment site (Wikel, 1982). The very high level of resistance observed after a single infestation is partially broken down by treating the host with histamine antagonists (Brossard, 1982; Wikel, 1982).

TICK MODULATION AND DEVIATION OF HOST IMMUNE DEFENSES

Tick countermeasures against host immune defences target those pathways shown to be important in acquisition and expression of acquired immunity (Wikel, Ramachandra & Bergman, 1994; Wikel, 1996). Modulation of host immune defences not only promotes successful blood feeding but it also enhances the ability of tick-borne pathogens to establish effectively in the host (Wikel, 1996, 1999; Schoeler & Wikel, 2001). An overview of tick modulation of host immune defences is provided in Table 1.

Antigen-specific T-cell activation triggers cytokine signalling pathways, cell differentiation and proliferation (Janeway *et al*. 2001). Examination of lymphocyte-elaborated cytokines provides insights into helper functions and cell mediated immune responses to infestation (Mosmann & Coffman, 1989). Tick infestation is often characterized by a reduction in the response of host lymphocytes to ConA and PHA stimulation, which is generally interpreted as an immunosuppressive phenomenon (Brossard & Wikel, 1997). This reduced response could be the result of decreased IL-2 production caused by tick saliva components like PGE_2 (Ribeiro *et al*. 1985; Inokuma, Kemp & Willadsen, 1994) or IL-2 binding proteins (Gillespie *et al*. 2001). PGE_2 primes naive T-cells for production of high levels of IL-4, IL-10 and IL-13, and very low levels of IL-2, IFN-γ, TNF-α, and TNF-β (Demeure *et al*. 1997; Harris *et al*. 2002). Peripheral blood lymphocytes of uninfested pure-bred *Bos indicus* and *B. taurus* were reduced in responsiveness to ConA when cultured in the presence of *D. andersoni* SGE (Ramachandra & Wikel, 1995). SGE or saliva of *I. ricinus* females inhibited proliferation (Mejri, Rutti & Brossard, 2002) and *in vitro* production of IL-2 by spleen lymphocytes from naive BALB/c mice stimulated with ConA (Ganapamo *et al*. 1996*a*). Those effects may be due to saliva immunosuppressive molecules and IL-10 production by T-cells (Ganapamo *et al*. 1996*b*). Modulation of host cytokine and T-cell proliferation has distinct advantages for the tick in regard to avoiding responses that would reduce the ability to obtain a blood meal. Altered host immune defences also provide an environment that is favourable for establishment of tick-transmitted infectious agents.

Guinea pigs infested with *D. andersoni* larvae were evaluated for their ability to develop a primary IgM antibody response to immunization with a thymic-dependent antigen, sheep red blood cells (SRBC), during tick exposure (Wikel, 1985). Antibody production was determined by the direct haemolytic plaque-forming cell assay. Infested animals produced significantly less anti-SRBC antibodies than uninfested controls. Ability to produce anti-SRBC antibodies returned to normal levels when animals were immunized with SRBC four days after termination of blood feeding. Similar results were obtained with mice infested with *I. ricinus* and immunized with SRBC (Mejri *et al*. 2002). Rabbits infested with adult *R. appendiculatus* were suppressed in their ability to develop an antibody response to bovine serum albumin when immunized during the peak of tick feeding (Fivaz, 1989). Decreasing the host antibody response during infestation can also reduce the potential for damage to the from anti-tick immunoglobulins or neutralization of tick saliva molecules needed for blood feeding.

Expression of IL-4 and IFN-γ mRNA in skin, draining lymph nodes and spleen was measured by competitive quantitative RT-PCR after infestation of BALB/c mice with 20 *I. ricinus* larvae (Lorimier & Brossard, personal communication). A peak of IL-4 mRNA was already is evident in the epidermis at 18 hours after the beginning of infestation, followed by an increase of IL-4 mRNA in the dermis 72 hours after infestation. An increase of IL-4 mRNA was concomitantly observed in spleen, and 24 hours later in lymph nodes draining the tick feeding site. IFN-γ mRNA consistently remained at low levels. Lymphocytes collected from draining lymph nodes of BALB/c mice infested with *I. ricinus* larvae, nymphs or adults produced high levels of IL-4 and low levels of IFN-γ after *in vitro* stimulation with tick saliva (Mejri *et al*. 2001) or with ConA (Ganapamo, Rutti & Brossard, 1995). These observations strongly suggest a Th2 polarization of the immune response. Infested mice developed IgE antibodies and intense immediate type hypersensitivity against tick antigens (Mbow *et al*. 1994). An increase of IL-5 and IL-10 was also observed in cultured CD4+ T cells stimulated with ConA (Ganapamo *et al*. 1996*b*). This polarized

cytokine pattern was also observed when BALB/c mice were infested with *A. hebraeum* or *B. microplus* larvae (Ganapamo, 1996). Draining lymph node cells from mice of different genetic backgrounds and infested with nymphs of *I. ricinus*, DBA (H-2d), C57BL/6 (H-2b), CBA (H-2k), C3H (H-2k), SJL (H-2s) and FVB (H-2q), produced more IL-4 than IFN-γ when stimulated *in vitro* with ConA (Christe, Rutti & Brossard, 1999). Lymph node cells from C3H/HeJ mice infested with *R. sanguineus* also developed a Th2 cytokine profile, represented by augmented IL-4, IL-10 and TGF-β and inhibited production of IL-2, IFN-γ and IL-12 (Ferreira & Silva, 1999). C3H/HeJ mice did not develop protection against *R. sanguineus*, as shown for BALB/c, C57BL/6 and C3H mice infested with *I. ricinus* nymphs (Christe *et al.* 1999). Likewise, C3H/HeN mice did not acquire resistance against nymphs of *I. scapularis* and *I. pacificus* (Schoeler *et al.* 2000). Cytokine production was biased toward a Th2 profile, with suppression of pro-inflammatory Th1 cytokines. Consequently, it seems that a Th2 immune response leads to a susceptible state in mice against almost all species of ticks.

SGEs of female *D. andersoni* collected daily over the course of feeding were assessed for their ability to alter the elaboration of cytokines by normal murine macrophages and T-cells stimulated with mitogens (Ramachandra & Wikel, 1992). Macrophage cytokine IL-1 elaboration was significantly suppressed by SGE. TNF-α production was also reduced. Macrophages collected from uninfested pure-bred *B. indicus* and *B. taurus* were also suppressed in their ability to elaborate IL-1 and TNF-α *in vitro* (Ramachandra & Wikel, 1995). T-cell elaboration of IL-2 and IFN-γ was inhibited and IL-4 production was not changed by the presence of SGE of *D. andersoni*. Expression of IFN-α, TNF-α, IL1α, IL-1β, IL-5, IL-6, IL-7 and IL-8 mRNA by human peripheral blood leukocytes were reduced when treated with a mixture of LPS and *R. appendiculatus* SGE (Fuchsberger *et al.* 1995). Exposure of a macrophage-like cell line to LPS and SGE of *R. appendiculatus* reduced both mRNA and secreted IL-1α, IL-10 and TNF-α (Gwakisa *et al.* 2001). The effects of repeated infestations of BALB/c mice with *D. andersoni* nymphs were studied in regard to lymphocyte proliferation, cytokine production and expression of adhesion molecules (Macaluso & Wikel, 2001). After two infestations, production of IL-2 was decreased but that of IFN-γ remained unchanged while the production of IL-4 and IL-10 was significantly enhanced. B-cell functions are also influenced by tick salivary gland molecules. *Ixodes ricinus* SGE inhibits B-cell production of IL-10 and expression of the early activation marker, CD69, on both B-cells and T-cells (Hannier *et al.* 2003). Expression of LFA-1 and VLA-4 by lymphocytes was suppressed. LFA-1 is particularly important in T-cell adhesion to endothelial cells and to APCs (Janeway *et al.* 2001). VLA-4 is upregulated following T-cell activation and is important for recruiting effector T-cells into sites of infection. Tick modulation of lymphocyte trafficking would be advantageous in reducing immune responses that could negatively impact blood feeding.

Although, there is extensive information about the effects of tick saliva or SGEs on host immune responses, little is known about the molecular mechanisms of those changes. A wide range of new proteins is expressed during blood feeding by *R. appendiculatus* females (Wang & Nuttall, 1994). Obviously, some of those molecules are essential for successful feeding. Studies indicate that some immunomodulation phenomena are induced by proteins. A 36 kDa soluble protein was found in saliva of female *D. andersoni*. This protein, for which the full-length gene sequence has been determined, suppressed *in vitro* proliferative response of murine splenocytes to ConA (Bergman, Ramachandra & Wikel, 1998; Bergman *et al.* 2000). The saliva of *I. scapularis* inhibited splenic T-cell proliferation and IL-2 secretion in response to ConA or PHA in a dose-dependent manner, while nitric oxide production by macrophages was diminished after stimulation by LPS (Urioste *et al.* 1994). A saliva protein of 5 kDa molecular weight or higher was responsible for those effects. An *I. scapularis* salivary gland protein designated Salp15 is an inhibitor of CD4+ T-cell activation by acting through repression of calcium-dependent signals initiated as a result of T-cell receptor engagement (Anguita *et al.* 2002).

As previously mentioned, genes are induced during the feeding process that result in the secretion of proteins involved in modulation of host immune and haemostatic responses (Leboulle *et al.* 2002*b*; Valenzuela *et al.* 2002). A 65 kDa immunogen was identified in saliva of feeding *I. ricinus* females (Ganapamo *et al.* 1997). The properties of another salivary gland protein induced during the *I. ricinus* feeding process, which is called Iris for '***I. r**icinus* **i**mmuno**s**uppressor', were characterized. The corresponding Iris mRNA sequence was first recovered by analysing a representational difference analysis subtractive library, and by using RACE methodology (Leboulle *et al.* 2002*b*). Immunoblot and confocal microscopy, using a specific antiserum, were used to demonstrate that Iris is secreted in saliva during blood feeding by female *I. ricinus* with an increasing expression of Iris from day three to day five of engorgement (Leboulle *et al.* 2002*a*). The effect of the corresponding recombinant protein (rIris/His) was studied in the context of human PBMCs cytokine production, using T-cells (PHA, ConA, CD3/CD28 and PMA/CD28), macrophages (LPS) and antigen presenting cell (PPD) activators. ELISA and ELISPOT assays showed that the

rIris/His suppressed the production of IFN-γ by human T-cells and APCs, while IL-5 and IL-10 levels remained unchanged. In contrast, rIris/His did not affect IFN-γ production, but it did enhance expression of IL-10 by macrophages. It was also shown that the expression of the pro-inflammatory cytokines IL-6 and TNF-α by macrophages, T-cells, and APCs was inhibited, while IL-10 expression remained unaffected. Furthermore, by neutralising rIris/His activity with a specific anti-rIris antibody, and by showing that purified rIris/His protein inhibited IFN-γ production by T-cells, it was clearly established that the recombinant protein contributed to the observed immunomodulation by inducing a Th2 type immune response. In addition, Iris modulated innate immune defences by inhibiting the production of pro-inflammatory cytokines (IL-6 and TNF-α).

Modulation of host immunity by tick saliva is of major importance both for successful blood feeding and for transmission of tick-borne pathogens. SGE of partially fed *D. reticulatus* females decreased natural killer cell activity *in vitro*, suggesting a mechanism that could potentiate transmission of some tick-borne viruses (Kubes *et al.* 1994). Inoculation of C3H/HeJ mice with a mixture of TNF-α, IFN-γ and IL-2 at the time of tick feeding suppressed *B. burgdorferi* transmission by *I. scapularis* (Zeidner *et al.* 1996). Th1 cytokines were down-regulated during the initial spirochete transmission period in C3H/HeJ infested with infected *I. scapularis* while IL-4 was augmented (Zeidner *et al.* 1997). Interleukin-4 produced following *I. ricinus* bites strongly influenced the antibody isotype response of BALB/c mice against *B. burgdorferi*, inhibiting specific anti-*B. burgdorferi* IgG2a (Christe, Rutti & Brossard, 2000). Transmission of *B. burgdorferi* and *Anaplasma phagocytophyla* by *I. scapularis* synergized to suppress splenic IL-2 production and diminish IFN-γ production (Zeidner *et al.* 2000). Splenic IL-4 production was increased after infestation of mice with co-infected ticks and levels of this cytokine were significantly higher than those induced by transmission of either pathogen individually.

GENOMIC AND PROTEOMIC STRATEGIES FOR ANALYSING TICK–HOST INTERACTIONS

Until recently, pharmacologically active tick salivary gland molecules have been identified and purified through a combination of fractionation methods, such as high performance liquid chromatography (HPLC), and biological assays to detect the activity of interest (Ribeiro & Mather, 1998). Biochemical purification methods are extremely effective, and a wealth of information has been obtained. This approach needs to be continued. However, powerful new methods exist for identifying the spectrum of genes expressed in the salivary glands of blood feeding arthropods (see chapter by Valenzuela in this Supplement). An expressed sequence tag (EST) is a partial nucleic acid sequence derived from clones selected at random from a cDNA generated from mRNA in a tissue of interest (Adams *et al.* 1991). Key to the utility of EST characterization is the body of gene sequences, translated amino acid sequences, and motifs recorded in public databases and available for comparison. Recently, the EST database (dbEST) was the most rapidly growing division of GenBank (Pandey & Lewitter, 1999). During the initial periods of analysis, many sequences from understudied organisms, such as ticks, will likely not have matches in the databases. As high throughput functional genomic methods for analysis of genes are developed, a more complete picture of tick salivary glands, or any other tissue of interest, will emerge (Valenzuela, 2002*a*).

The first reported tick EST project was based on a cDNA library prepared from whole larvae of *B. microplus* (Crampton *et al.* 1998). That library was pre-screened against housekeeping genes that were frequently encountered during the early period of sequencing. A total of 234 unique ESTs were identified; 142 of them were not previously identified in arachnids. Database matches were not found for 39% of the total ESTs. Whole *A. americanum* larvae and adults were used to construct cDNA libraries, and 1462 adult and 480 larval ESTs were sequenced (Hill & Gutierrez, 2000). Only 44·4% of ESTs were tentatively identified based on translated amino acid sequences matched in the public databases. An important point to keep in mind is that a database match does not necessarily assure that the functions of the two proteins are similar.

Random sequencing was carried out on clones of an *I. scapularis* full length cDNA library prepared from females feeding for three to four days (Valenzuela *et al.* 2002). A total of 735 clones were sequenced and grouped into 410 clusters of which 383 were not previously associated with *I. scapularis*. In the same study, salivary gland homogenate and saliva were fractionated by SDS–PAGE and protein bands were subjected to N-terminal amino acid sequencing (Valenzuela *et al.* 2002). Often two-dimensional gel protein spots might not yield sufficient material for N-terminal amino acid sequencing. When SDS–PAGE can be effectively used, as in the report by Valenzuela *et al.* (2002), the likelihood of having sufficient protein for analysis is greater. Those amino acid sequences were then matched to translated sequences of clones from the salivary gland cDNA library. These combined approaches provide valuable insights into families of proteins expressed in the salivary gland of the feeding tick. Subtractive and full length cDNA libraries were prepared from mRNA isolated from five-day fed female *I. ricinus* and analysed for homologies to genes of recognised function (Leboulle *et al.* 2002*b*). Clones were found with high homologies with anti-coagulants, platelet

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aggregation inhibitors, and immunomodualtors. In addition, genes were cloned and expressed that encoded putative metallopeptidase, elastase inhibitor and human tissue factor pathway inhibitors (Leboulle *et al*. 2002*b*). Differential display was used to compare genes expressed in the salivary glands of male *A*. *americanum* and male *D*. *andersoni*, resulting in the identification of several genes encoding molecules with house keeping functions as well as a histamine binding protein (Bior, Essenberg & Sauer, 2002).

Combined genomic, functional genomic, and proteomic analyses will provide rapid insights into the complex pharmacology of tick saliva and the relationship of those molecules with host immune responses and pathogen transmission. High throughput screening of genes and the proteins they encode are essential considerations for moving these studies forward (Valenzuela, 2002*b*). A key element will be identification of biological functions of those numerous gene products that do not currently have matches in the public databases. Those molecules are likely to be important to the unique lifestyles of ticks. The recent demonstration that RNA interference (RNAi) can be effectively used in ticks provides a valuable tool for identification and significance of gene function through gene silencing (Aljamali *et al*. 2003). RNAi has emerged as a widely used tool (Denli & Hannon, 2003), which will certainly be increasingly applied to arthropod vectors of disease. Most certainly, a tick genome sequencing project will be initiated in the near future, which will provide information of vast importance in understanding tick biology, pathogen transmission and new avenues for control.

FUTURE DIRECTIONS

Gene discovery projects will identify *in silico* molecules of potential importance in modulating host immune defences and facilitating transmission of infectious agents. Once those molecules are cloned and expressed, often preferably in eukaryotic expression systems, their biological activities can be determined. EST and genome studies have the potential to enhance dramatically the pace of discovery of relevant molecules. The role of prostaglandins in tick modulation of host immune defences needs to be definitively determined. A major focus should be determining the mechanisms (signaling pathways, receptor–ligand interactions, activation and regulation of cellular interactions) of acquired resistance and tick circumvention of host immune defenses. How are the profiles of tick salivary gland secretions altered during the course of tick infection and transmission of infectious agents and what are the biological implications of any observed differences? Determine the molecular diversity in salivary gland secretions within and among ticks species.What are the contributions to acquired resistance of dendritic cells, NK cells, gamma/delta T-cells, keratinocytes, and cytotoxic T-cells? How are the activities of those cells impacted by tick salivary gland molecules? What are the cellular and molecular interactions among host, tick and infectious agent at the bite site? Identify salivary gland molecules that can be incorporated into transmission blocking vaccines. Our understanding of tick immunobiology has increased dramatically during the past 25 years. We are poised to make rapid advances in the coming years, which will greatly enhance our understanding of the tick–host interface, and those advances have the potential to improve the quality of life for humans and other animal species.

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