IMMUNOLOGY OF THE HOST-VECTOR-PATHOGEN INTERFACE

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Hematophagous arthropods and the diseases they transmit are of vast medical and veterinary public health importance. Ticks are the most important vectors of pathogens to domestic and wild animal species, and they are second only to mosquitoes as transmitters of disease causing agents to humans.[1] The patterns, number and variety of tick-borne diseases continues to change.[2] It is likely that this trend will continue into the future. This review will focus upon tick-host-pathogen interactions, due to the body of information available related to these associations.

Pathogens often undergo complex developmental cycles within the vector.[3] The dynamic nature of host-vector-pathogen interactions is emerging. New knowledge gained from the analysis of these relationships can result in development of effective strategies for suppression of disease transmission, provide enhanced understanding of pathogen development, pathogenesis, host responses to the disease causing agent and reactivity to the vector.

Worldwide incidence of Lyme borreliosis is increasing.[4,5] Ehrlichiosis is emerging as a tick-borne disease of increasing human and veterinary importance.[6] Anaplasmosis is an arthropod-borne rickettsial disease of cattle and other ruminants, which causes vast losses in meat, milk and fiber production.[7] Significant gaps exist in our knowledge of these and other tick-borne diseases in regard to transmission dynamics, development within the vector, pathogenesis and host responses to infection.

Estimated economic impact of ticks and the diseases they transmit is estimated to be at least seven billion dollars.[8] Losses attributable to ticks, and tick-borne pathogens, are a major constraint on agricultural development in large areas of the tropical and sub-tropical world. Effective tick control would result in significantly improved quality of life in those geographic areas.

Control of ticks is almost totally dependent upon use of acaricides, and ixodid resistance to these chemicals represents a serious threat to animal health and production on a global scale.[9] Cost of research and development of new acaricides, rapid onset of resistance to those formulations and reduced potential for economic return are factors, which discourage the investments necessary to discover, license and market new compounds.[10] Increasing public concern about chemicals as residues in food, their impact upon human health and the environment are factors driving the need to develop new approaches to vector control. Considerable attention has been focused upon the development of anti-tick vaccines.[11] A

thorough understanding of host immunity to tick infestation is essential for design of an effective immunological based control strategy.

HOST IMMUNITY TO THE TICK

Laboratory animals and bovines acquire resistance to tick feeding as a consequence of infestation, and the immune responses involved in acquired immunity have been investigated.[11, 12, 13, 14] Expression of acquired resistance results in reduced numbers of engorged ticks, decreased blood meal volume, impaired production and reduced viability of ova, increased duration of engorgement, impaired transmission of pathogens and tick death.

The cellular infiltrate at the bite site during an initial infestation is minimal; however, guinea pigs and cattle expressing acquired resistance to infestation develop cutaneous basophil hypersensitivity reactions at tick attachment sites.[13, 15, 16] These basophil rich reactions appear to be directly linked to damage and rejection of the feeding tick.[17] Basophils were observed within injured tick digestive tract epithelial cells.

The host immunological mechanisms involved in acquisition and expression of tick resistance include: circulating and homocytotropic antibodies, complement, cell mediated effector elements, cytokines and other mediators of the inflammatory response.[11, 12, 13, 14, 18, 19] Upon attachment to the host a complex and changing array of salivary gland derived molecules are introduced into the bite site.[20] These molecules are trapped by Langerhans cells[21] and presented to host B and T-lymphocytes. Host cytokines play an important role in communication among antigen presenting cells and immunocompetent lymphocytes.[19] The question of whether or not host cytokines directly impact upon the tick and tick-borne pathogens remains to be determined.

Primary antibody and cell mediated immune responses are stimulated, during the course of feeding. B and T-lymphocyte memory cells specific for tick salivary antigens are generated. The full force of the primary immune response to feeding is likely not expressed until after the ticks have engorged. Observed cellular infiltrate at this time could result from activation of the alternative complement pathway and the degranulation of cutaneous mast cells. The stage is set for a secondary immune response upon continued, or repeated, exposure to tick salivary immunogens.[11, 12, 13, 14]

Expression of acquired resistance can have a rapid, dramatic impact upon attached ticks.[13, 14] Salivary immunogens are introduced into the host and processed by antigen presenting, Langerhans cells. Reactive new and memory B and T-lymphocyte clones are stimulated. The "secondary response" is more rapid and intense than the reactivity to an initial infestation. Tick specific, preformed, immunoglobulins might still be in the circulation and basophils could be "armed" with homocytotropic antibodies bound to their Fc receptors. Cell mediated reactivity is evident due to the rapid onset of the cutaneous basophil hypersensitivity response. Activation of complement through the alternative and/or classical pathways would result in the generation of a variety of biological

activities, including the anaphylatoxins C3a and C5a. Chemotactic moieties would enhance the development of the cutaneous lesions and rejection response. Target immunogens involved in tick rejection have not been clearly defined. The possible role, if any, of cytotoxic T-lymphocytes in tick rejection has not been determined.

TICK SALIVA AND HOST IMMUNOSUPPRESSION

Saliva of ixodid ticks changes in composition during the process of engorgement[20], and it contains a variety of immunogens and pharmacologically active molecules.[11, 22, 23, 24]. Alteration of host defenses and inflammatory responses provides an environment, which facilitates tick feeding and pathogen transmission. Saliva of Ixodes dammini contains anti-inflammatory, anti-hemostatic and immunosuppressive factors.[25] Production of interleukin-2, IL-2, by T-lymphocytes was inhibited by salivary prostaglandin E_2.[25]. The alternative pathway of complement activation was shown to be inhibited by a 49 kilodalton, kdal, molecule in the saliva of I. dammini[22], and host neutrophil function is altered by tick saliva.[26] The alternative pathway of complement was found to be important in the expression of acquired anti-tick resistance.[27] This complement pathway could be involved in a variety of activities, including chemoattraction of cells to the bite site. Dermacentor andersoni salivary glands contain antagonists to host coagulation factors V and VII.[28] Blood flow is increased at the feeding site and platelet aggregation is inhibited.[23] These factors facilitate engorgement and inhibit the host immune response to the vector and any pathogens, which might be introduced into the host.

Tick feeding reduces the ability of host derived lymphocytes to proliferate in vitro to T-cell mitogens[29], and to develop a primary antibody response to a thymic dependent antigen.[30] Salivary gland extracts prepared on days zero through nine from unmated, female D. andersoni were tested for their ability to influence cytokine production by normal macrophages and lymphocytes and to alter lymphocyte in vitro responsiveness to B and T-cell mitogens.[19] In vitro proliferation of normal lymphocytes to the B-cell mitogen Escherichia coli lipopolysaccharide was enhance, while responsiveness to the T-lymphocyte mitogen, concanavalin A was suppressed by up to 68.4 percent. These results confirmed the reactivity patterns of lymphocytes obtained from infested animals and exposed in vitro to the same mitogens.[19, 29]

Cytokines are critical components in the regulation of the immune response.[19] cytokines evaluated were interuleukin-1 and tumor necrosis factor alpha, produced by macrophages, and interleukin-2 and gamma interferon, produced by T-lymphocytes. Interleukin-1 elaboration was significantly suppressed by salivary gland extracts prepared during the first five days of engorgement by up to 89.8 percent. Tumor necrosis factor alpha was suppressed by salivary gland extracts for each day studied by up to 94.6 percent. Interleukin-2 production was suppressed by up to 31.9 percent and gamma interferon levels were reduced to a maximum of 57.0 percent. These cytokines have a variety of functions in regulation of host inflammatory and immune responses. Both tumor necrosis factor alpha and gamma interferon possess anti-viral

properties and induce expression of class I and II major histocompatibility complex, MHC, molecules. Class II MHC molecules are critical for presentation of antigen to immunocompetent lymphocytes, and class I MHC molecules associate with antigens for effective targeting of cytotoxic T-lymphocytes. Interleukin-2 has an autocrine role in T-lymphocyte function. Reduced cytokine levels during tick feeding would clearly have the potential of impairing the acquisition and expression of host acquired resistance to infestation or any transmitted pathogen. Tick induced immunosuppression is not so profound as to result in a total loss of host immune competence, which would could lead to an overwhelming infection by introduced pathogens. As in any well established host-parasite relationship, a balance exists between the physiological integrity of the host and the ability of the pathogen to become established.

IMPACT OF HOST IMMUNITY TO THE ARTHROPOD ON PATHOGEN TRANSMISSION

Several investigations have provided evidence that host immune reactivity to the arthropod vector altered transmission of vector-borne pathogens.[13] Immunization of mice with normal mosquito salivary glands protected mice against an intraperitoneal challenge with the rodent malarial parasite, Plasmodium berghei.[31] Antibodies to Aedes aegypti were found to neutralize mosquito propagated Sindbis virus, but not virus grown in Vero cells.[32] Mosquito salivary gland antigens clearly stimulated host immune responses, which reacted with a mosquito-borne protozoan and virus. Target immunogens and the role of such responses in nature have not been established.

Host acquired resistance to infestation with pathogen-free D. andersoni altered the ability of that ixodid species to transmit the highly virulent tick-borne bacterium, Francisella tularensis.[33] All control rabbits, not resistant to D. andersoni infestation, developed F. tularenis infection. Only 34.4 percent of rabbits expressing acquired resistance to the tick became infected and died. None of the remaining tick resistant animals developed antibodies reactive with F. tularensis. Host acquired resistance to the bite of a pathogen-free tick induced a response, which altered the ability of a highly virulent bacterial pathogen to be transmitted and/or establish within a host. The mechanism(s) responsible for observed protection is(are) unknown.

Similar epitopes are expressed by both microfilariae of Brugia malayi and the mosquito vector, A. aegypti.[34] The shared antigens expressed by B. malayi are not likely due to adsorbed mosquito vector epitopes, since the microfilariae are produced from female worms within the host. However, the impact of any reactivity to the shared epitopes upon uptake of the microfilariae by the mosquito vector is not known. It is possible that the shared epitopes are important in survival of the helminth within the mosquito.[34]

Host-vector-pathogen interactions are complex. The vector has the ability to alter the immune competence of the host by introduction of immunosuppressive factors. These elements could clearly enhance vector feeding and pathogen establishment.

Salivary gland lysates of the sandfly Lutzomyia longipalpis substantially enhanced the infectivity of Leishmania major for mice.[35] Infection with low numbers of protozoa could only be established in the presence of salivary gland material. Host immunity to the vector clearly can impact upon the vector-borne pathogen. It is possible that host immunity to the vector neutralizes salivary gland factors, which promote pathogen establishment. This could explain how host immunological reactivity to salivary secretions of a pathogen-free vector could reduce the infectivity of a pathogen. Experiments need to be performed to address this topic.

IMMUNOLOGICAL CONTROL OF ARTHROPODS

Previously stated problems associated with the use of acaricides[9] indicate the need for alternative methods of tick control. A promising approach is the development of anti-tick vaccines.[11,36] Initial efforts to develop immunization regimens for the induction of immunity to ticks used extracts of whole ticks or salivary glands.[11, 12, 13] These extracts contained numerous immunogenic molecules and the resistance induced was often of variable intensity. Emphasis has now shifted to the use of Immunogens not introduced into the host during the course of tick feeding. These "concealed" or "novel" immunogens are derived from tick digestive tract[1, 36, 37, 38] or integument.[39]

Immunization with digestive tract immunogens has been shown to be an effective way to induce laboratory animal and bovine be an effective way to induce laboratory animal and bovine resistance to tick feeding.[11, 36, 37, 38] Host antibodies and other immune effector elements to tick gut are contained within the blood meal. As the tick takes up blood from an immunized animal, host antibodies, complement and possible effector cells damage the gut of the tick. The end result is reduced engorgement, reduction or prevention of egg production and death of the feeding tick often occurs. The tick is exposed to a large amount of host blood during engorgement. An unfed female ixodid tick weighing ten milligrams might reach a final engorged wight of 1000 milligrams; however, she would have processed approximately 4000 milligrams of blood, during feeding.[40] An advantage of immunization with tick gut derived epitopes is the absence of priming for cutaneous hypersensitivity to salivary gland epitopes introduced during feeding.

Cattle have been successfully vaccinated with a membrane derived 89 kdal glycoprotein obtained from semi-engorged female Boophilus microplus.[36, 41] Protection inducing immunogen was present in very low quantities, and only microgram amounts were needed to induce significant resistance to infestation, which reduced engorgement, ova production and tick survival. Immunoglobulins obtained from successfully immunized animals inhibited enocytotic activity of tick digestive tract cells.[41] Protection inducing 89 kdal molecule was subsequently cloned and expressed by E. coli as a fusion protein, which could be used to induce resistance to infestation.[42] An important consideration in the development of any vaccine, particularly for recombinants, is the inclusion of epitopes broadly recognized by T-lymphocytes.[43] Since a vaccine

will be administered to a genetically diverse, randomly bred, population of animals, it is essential to have epitopes that can be effectively presented in association with class II MHC to immunocompetent lymphocytes by antigen presenting cells.

Hereford steers have been successfully immunized with membrane enriched extracts and 100,000 x g supernatant of B. microplus midgut.[37] Three protection inducing immunogens were found in the detergent solubilized membrane fraction.[38] The exact nature of these immunogens remains to be reported.

Greater than 50 percent of sera derived from mice infested with Ixodes ricinus nymphs and rabbits infested with I. ricinus adults reacted with a 25 kilodalton integumental immunogen.[39] These antibodies cross-reacted with a 20 kdal molecule in the integument of Rhipicephalus appendiculatus.[39] A vaccine directed against accessible integumental epitopes should be considered for further investigation.

Cells of a primary in vitro culture of developing larvae of Amblyomma americanum were used to induce resistance to infestation with adults of the same species.[44] Mean engorgement weight of female A. americanum, which obtained a blood meal from vaccinated animals, was reduced by a mean of 74.8 percent and 54.6 percent of female ticks feeding upon immunized animals, died.[44] Animals immunized with A. americanum, which obtained a blood meal from vaccinated animals, was reduced by a mean of 74.8 percent and 54.6 percent of female ticks feeding upon immunized animals, died.[44] Animals immunized with A. americanum primary tissue culture cells were also resistant to challenge infestation with D. andersoni females, reducing mean engorgement by 71.3 percent.[44] Cross-protection would indicate the presence of share epitopes, which could possibly be used to develop a vaccine with the ability to induce protection against widely divergent ixodid species. Function and localization of these epitopes have not been determined.

Anti-tick vaccine research in this laboratory involves the identification, isolation and characterization of tick digestive tract brush border molecules that can be used as effective protection inducing immunogens.[11] Animals immunized with gut absorptive surface fragments developed a strong resistance to infestation with adult females of the homologous species. Mean weight of blood meal obtained was reduced by 69.8 percent and up to 71.5 percent of the challenge ticks died. An important observation was the absence of cutaneous hypersensitivity reactions at tick attachment sites on immunized animals.

Sera of immunized animals displaying resistance to infestation were used to identify reactive gut components by immunoblotting.[11] Gut derived molecules were separated by one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroeluted to nitrocellulose. Molecular weights of reactive bands were 64, 66, 68, 113, 125, 133, 137, 141, 144, 153 and 157 kdal. Control animals neither vaccinated nor exposed to tick infestation had immunoglobulins reactive with a 61 kdal polypeptide.

Similar nitrocellulose immobilized electrophoretograms were reacted with biotinylated lectins in order to determine the

presence of glycoconjugates, since lectin affinity chromatography could be a possible first purification step. Vast majority of gut derived molecules were determined to be glycoconjugates. Lectin blot and immunoblot reactivities were compared, and it was found that the combined specificities of peanut agglutinin and wheat germ agglutinin complexed with all the same bands on the electrophoretogram as did the antibodies derived from successfully vaccinated animals.[11]

The complexity of these studies is increased due to the need to screen each fraction for the ability to induce anti-tick immunity. Each immunized animal must then be analyzed in regard to specificity of antibody response by enzyme-linked immunosorbent assay and immunoblotting. Cell mediated immune reactivity is assessed by antigen specific in vitro lymphocyte proliferative responses to components of the immunogen preparation. In this way, protection inducing fractions with both B and T-lymphocyte reactivity can be identified.

Purification of immunogens is achieved through: differential centrifugation of tick gut absorptive surface membranes, solubilization with non-ionic detergents, gel filtration high performance liquid chromatography, separation based upon isoelectric point of molecules of interest and immunoaffinity chromatography with immobilized monoclonal immunoglobulins reactive with molecules of interest. A variety of contemporary biotechnological approaches can be used to obtain sufficient quantities of immunogens of interest. Vaccine could be administered in an injectable slow release formulation, which would require minimal handling of cattle for immunization.

Anti-arthropod vaccines will not be limited to long-term blood feeders. Species of particular interest would be mosquitoes, stable flies, horn flies, sucking lice and mange mites. Feasibility of anti-arthropod vaccines is clearly established.[11, 36] Anti-arthropod immunization based control will likely find a place in integrated vector suppression strategies. The technology is at hand to proceed with development of these vaccines.

ACKNOWLEDGEMENTS

Research of the authors is supported by U.S. Department of Agriculture Grants 88-34166-3759 and 88-34116-4206, the Oklahoma Center for the Advancement of Science and Technology and by an award from the SmithKline Beecham Animal Health. This manuscript is Oklahoma Agricultural Experiment Station publication number: 6250.

REFERENCES

1. Balashov, Y.S. Bloodsucking ticks (Ixodoidea) - vectors of disease of man and animals. Miscellaneous Publications of the Entomological Society of America. 1972; 8:161-376. 2. Hoogstrall, H. Changing patterns of tickborne disease in modern society. Annual Review of Entomology. 1981; 26:75-99. 3. Kocan, K.M., Hair, J.A. and Ewing, S.A. Ultrastructure of Anaplasma marginale Theiler in Dermacentor andersoni Stiles and Dermacentor variabilis

(Say). American Journal of Veterinary Research. 1980; 41:1966-1976. 4. Steere, A.C. Lyme disease. New England Journal of Medicine. 1989; 321:586-596. 5. Szczepanski, A. and Benach, J.L. Lyme borreliosis: Host responses to Borrelia burgdorferi. Microbiological Reviews. 1991: 55-21-34. 6. Magnarelli, L.A. Ehrlichiosis: A veterinary problem of growing epidemiologic importance. Clinical Microbiology Newsletter 1990; 12:145-147. 7. McCallon, B.R. Prevalence and economic aspects of anaplasmosis. In, Proceedings of the Sixth National Anaplasmosis Conference. E.W. Jones, editor. Heritage Press. Stillwater, Oklahoma. 1973; pp. 1-3. 8. Food and Agricultural Organization (of the United Nations). Ticks and tick-borne disease control. A practical field manual. Rome. Food and Agricultural Organization. 1984. 9. Solomon, K.R. Acaricide resistance in ticks. Advances in Veterinary Science and Comparative Medicine. 1983; 27:273-296. 10. Durand, M.R.E. Ticks - a warning. Queensland Agriculture Journal. 1976; pp. 541-544. 11. Wikel, S.K. Immunological control of hematophagous arthropod vectors: Utilization of novel antigens. Veterinary Parasitology. 1988; 29:235-264. 12. Willadsen, P. Immunity to ticks. Advances in Parasitology. 1980; 18:293-313. 13. Wikel, S.K. Immune responses to arthropods and their products. Annual Review of Entomology. 1982; 27:21-48. 14. Wikel, S.K. Immunomodulation of host responses to ectoparasite infestation - an overview. Veterinary Parasitology. 1984; 14:321-339. 15. Allen, J.R. Tick resistance: basophils in skin reactions of resistant guinea pigs. International Journal for Parasitology. 1973; 3:195-200. 16. Allen, J.R., Doube, B.M. and Kemp, D.H. Histology of bovine skin reactions to Ixodes holocyclus, Neuman. Canadian Journal of Comparative Medicine. 1977; 41:26-35. 17. Voss-McCowan, M.E. Changes in the midgut of female Amblyomma Americanum as a result of feeding upon hosts expressing different levels of acquired anti-tick resistance. Ph.D. dissertation. University of North Dakota. 1991; pp. 310. 18. Brossard, M., Rutti, B. and Haug, T. Immunological relationships between hosts and ixodid ticks. In, Parasite-Host Association: Coexistence or Conflict. C.A. Toft, A. Aeschliman and L. Bolic, editors. Oxford Science Publication. 1991; pp. 177-200. 19. Ramachandra, R.N. and Wikel, S.K. Modulation of the host immune response by ticks (Acari: Ixodidae): Impact of salivary gland extracts on host macrophage and lymphocyte cytokine production. Journal of Medical Entomology. 1992; In Press. 20. Oaks, J.F., McSwain, J.C., Bantle, J.A., Essenberg, R.C. and Sauer, J.R. Putative new expression of genes in ixodid tick salivary gland development during feeding. Journal of Parasitology. 1991; 77:378-383. 21. Nithiuthai, S. and Allen, J.R. Significant changes in epidermal Langerhans cells of guinea pigs infested with ticks (Dermacentor andersoni). Immunology. 1984; 51:133-141. 22. Ribeiro, J.M.C. Ixodes dammini: salivary anti-complement activity. Experimental Parasitology. 1987; 64:347-353. 23. Ribeiro, J.M.C. Role of saliva in blood-feeding by arthropods. Annual Review of Entomology. 1987; 32:463-478. 24. Ribeiro, J.M.C., Evans, P.M., McSwain, J.C. and Sauer, J.R. Amblyomma americanum: characterization of salivary prostaglandins E_2 and F_2 alpha by RP-HPLC/bioassay and gas chromatography - mass spectrometry. Experimental Parasitology. 1992; 74:112-116. 25. Ribeiro, J.M.C.,

Makoul, G.T., Levine, J., Robinson, D.R. and Spielman, A. Antihemostatic, antiinflammatory and immunosuppressive properties of the saliva of a tick, Ixodes dammini. Journal of Experimental Medicine. 1985; 161:332-344. 26. Ribeiro, J.M.C., Weis, J.J. and Telford, S.R.., III. Saliva of the tick Ixodes dammini inhibits neutrophil function. Experimental Parasitology. 1990; 70:382-388. 27. Wikel, S.K. Acquired resistance to ticks. Expression of resistance by C4-deficient guinea pigs. American Journal of Tropical medicine and Hygiene. 1979; 28:586-590. 28. Gordon, J.R. and Allen, J.r. Factors V and VII anticoagulant activities in the salivary glands of feeding Dermacentor andersoni ticks. Journal of parasitology. 1991; 77:167-170. 29. Wikel, S.K. Influence of Dermacentor andersoni infestation on lymphocyte responsiveness to mitogens. Annals of Tropical Medicine and Parasitology. 1982; 76:627-632. 30. Wikel, S.K. Effects of tick infestation on the plaque-forming cell response to a thymic dependent antigen. Annals of Tropical Medicine and Parasitology. 1985; 79:195-198. 31. Alger, N.E., Harant, J.A., Willis, L.C. and Jorgensen, G.M. Sporozoite and normal salivary gland induced immunity in malaria. Nature. 1972; 238-341. 32. Feinsod, F.M., Spielman, A. and Waner, J.L. Neutralization of Sindbis virus by antisera to antigens of vector mosquitoes. American Journal of Tropical Medicine and Hygiene. 1975; 24:533-536. 33. Wikel, S.K. Host resistance to tick-borne pathogens by virtue of resistance to tick infestation. Annals of Tropical Medicine and Parasitology. 1980; 74:103-104. 34. Lastre, M., Cesbron, J.Y., Perez, O., Henry, D., Ouaissi, Dissous, C. and Capron, A. Brugia malayi microfilariae share epitopes with Aedes aegypti. Parasitology. 1988; 97:247-254. 35. Titus, R.G. and Ribeiro, J.M.C. The role of vector saliva in transmission of arthropod-borne disease. Parasitology Today. 1990; 6:157-160. 36. Willadsen, P., McKenna, R.V. and Riding, G.A. Isolation from the cattle tick, Boophilus microplus, of antigenic material capable of eliciting a protective immunological response in the bovine host. International Journal for Parasitology. 1988; 18:183-189. 37. Opedebeeck, J.P., Wong, J.Y.M., Jackson, L.A. and Dobson, C. Hereford cattle immunized and protected against Boophilus microplus with soluble and membrane-associated antigens from the midgut of ticks. Parasite Immunology. 1988; 10:405-410. 38. Wong, J.Y.M. and Opdebeeck, J.P. Protective efficacy of antigens solubilized from gut membranes of the cattle tick, Boophilus microplus. Immunology. 1989; 66:149-155. 39. Rutti, B. and Brossard, M. Repetitive detection by immunoblotting of an integumental 25 kDA antigen in Ixodes ricinus and a corresponding 20-kDa antigen in Rhipicephalus appendiculatus with sera of pluriinfested mice and rabbits. Parasitology Research. 1989; 75:325-329. 40. Kaufman, W.R. Tick-host interaction: A synthesis of current concepts. Parasitology Today. 1989; 5:47-56. 41. Willadsen, P., Riding, G.A., McKenna, R.V., Kemp, D.H., Tellam, R.L., Nielsen, J.N., Lahnstein, J.,Cobon, G.S. and Gough, J.M. Immunologic control of a parasitic arthropod. Identification of a protective antigen from Boophilus microplus. Journal of Immunology. 1989; 143:1346-1351. 42. Rand, K.N., Moore, T., Sriskantha, A., Spring, K., Tellam, R., Willadsen, P. and Cobon, G.S. Cloning and expression of a protective antigen from the cattle tick Boophilus

microplus. Proceedings of the National Academy of Sciences, U.S.A. 1989; 86:9657-9661. 43. Sher, A. Vaccination against parasites: special problems imposed by the adaptation of parasitic organisms to the host immune response. In, The Biology of Parasitism. Alan R. Liss, Inc., New York. 1988; pp. 169-182. 44. Wikel, S.K. Resistance to ixodid tick infestation induced by administration of tick-tissue culture cells. Annals of Tropical Medicine and Parasitology. 1985; 79:513-518.