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Host Skin during Tick Infestation: Unearthing the Local Immunity to Lead an Anti-Tick Vaccine

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ABSTRACT

***Background*:** The host skin is the first line of defense against most microorganisms and parasites such as bacteria, fungi and ticks. The immune system present in skin takes part of a sophisticate defense mechanism, firstly as physical, cellular and chemical barriers, followed by a wide range of antimicrobial molecules and specialized immune cells. These cells are responsible for inflammatory processes, antigen uptake and presentation, allergic responses that untimely could control the pathogens.
***Review*:** Concerning tick parasitism, skin immunity has a paramount role during tick attachment and blood feeding through both the innate and adaptive responses. In recent years, an increasing number of discoveries in tick physiology revealed a more detailed picture of the role of immune cells and their mediators against tick parasitism. Therefore, a systematic review and summarization of this information can give a more comprehensive understanding of the orchestration of the diverse and complex host immune response mechanisms that reject at least part of infesting ticks and give clues to suggest potential applications to develop better methods for tick control.
***Conclusion*:** The local skin immune response to tick and other ectoparasite infestations is intricately influenced by the microenvironment created by parasite attachment components and secreted proteins, attracting and engaging local immune cells. Host immune status further contributes to this dynamic. This review discusses the major cellular responses, functional diversity, and host skin immunity mechanisms stimulated by ticks. However, more research is needed to fill existing gaps and fully understand how the skin responds to ticks and other parasites. For example, studying B-cell responses, their diversity, and exploring the full Th2 immune response could provide valuable insights for improving tick control strategies.

Keywords: tick, skin, immunity, parasite, vaccine.

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I. INTRODUCTION

1. The expanding threat of ticks

Ticks are obligate blood-feeding ectoparasites that target both humans and a diverse array of domestic and wild animals [30]. Ticks serve as vectors for a wide range of organisms, including parasites, viruses, and bacteria, triggering numerous tick-borne diseases, someof which are highly serious [28]. Found globally, ticks pose a significant threat to animal husbandry, as well as to humans, emerging as a major challenge in the global control of infectious and parasitic diseases [30]. Despite extensive global efforts over more than a century to control or eliminate ticks and mitigate their direct and indirect effects [32,33,42,65,129,137], outbreaks of tick populations in previously tick free areas are rising. Examples include *Haemaphysalis longicornis* in the United States [123], and *Rhipicephalus microplus* in East African countries [84]. Additionally, ticks have also been found infesting and adapting to new host species in different regions, expanding the scope of tick host accidental parasitism [75].

The spread of Lyme disease to new areas in the north hemisphere increased due to climate changes [104], further exacerbates the challenge. The expanding distribution, coupled with adaptation to new hosts and factors like drug resistance, worsens the difficulty in controlling tick populations. Consequently, ticks present an enormous challenge to both public health and animal production. For example, this impact is evident in North America with *Ixodes scapularis* and *Ixodes pacificus* population as well as in Europe and Asia with, *Ixodes ricinus* and *Ixodes persulcatus*, all competent vectors of *Borrelia burgdorferi* the agent of Lyme disease [79,93]. Considering these challenges, there is a clear need for strategic improvements in methods to control ticks and tick-borne diseases contemplating the diversity of tick species and their expanding range of hosts.

2. Host immune resistance against ticks

The hematophagous feeding mechanisms of ticks have evolved over circa 100 million years [67,77]. Hard ticks feed on the host for several days at the attachment site, ingesting a volume of blood that increases their body weight by hundreds of times [9]. In contrast, soft ticks feed faster, taking minutes to hour, and increase their initial body weight by 2 to 10 times [76,77]. It is not surprising that ticks have developed various adaptations to modulate the host immune system enabling specific blood-feeding behaviors [110]. Indeed, ticks have evolved to inject into the host an arsenal of molecules that inhibit host wound healing, hemostasis, inflammation, cell proliferation and tissue remodeling [23,90], employing distinct mechanisms of action [100]. Given that hosts hemostatic systems emerged before the tick anti-hemostatic mechanisms [77], it is remarkably interesting, although complex, to understand the parasite-host relationship in different environments.

The understanding skin innate and adaptive cellular immune responses against ticks remain incomplete or totally unknown for most host species [61,91]. Immune mechanisms, such as physical and chemical barriers in the skin, inducible mechanisms that are amplified by the engagement of pattern recognition receptor (PRRs), and lymphocytes populations in the skin are crucial to elucidate [138]. These mechanisms remain largely unknown due to limited study during tick parasitism. Although, it is now clear that they have a relevant role in the interaction between the diverse range of ticks, hosts, and microorganisms, therefore resulting in complex interactions [47,48,66,95,118]. In this sense, despite progress in the search of tick molecules suitable to use as vaccine antigens, the lack of high protection levels from such antigens is hindered

by the lack of a clear understanding of cells and components of the innate and adaptive immune response in tick-host interaction [2,29,41]. The immunological control strategies, based on the use of vaccines have focused for many decades on the empirical selection and characterization of suitable tick proteins [1,49]. Additionally, these vaccines provide an even more limited protection level when used against multiple tick species or different tick populations of the same species [96]. Despite promising results shown by potential antigens in recent years [85], the goal of achieving an effective universal anti-tick vaccine remains elusive.

Further studies on the interaction between ticks and their hosts are essential to understand function of tick salivary gland, blood digestion, and pathogen transmission [23,70]. Accordingly, understanding details of specific immunological mechanisms against ticks in host tissues is crucial for the development of more effective strategies in anti-tick control [81]. While typical macroscopic and microscopic characteristics associated with the tick bite site are well established in some tick-host infestation models [8,10,17,20,22,39,44,50-52,54,70,97,103], molecular and cellular determinants for tick resistance at the tick attachment tissues in the natural host have not been adequately explored, hindering the development of effective anti-tick vaccines.

3. Distinct degree of resistance against ticks among hosts

Various methodologies and analyses have been employed to determine the immunological responses that hosts deploy against ticks (Table 1). The idea of immune protection against tick traces back to Trager [121], who demonstrated that rabbits repeatedly infested with ticks were able to partially impair the parasite reproduction. This scientific endeavor continually demonstrates that not only hemostatic, humoral, and systemic immune responses are important, but a local immune response also plays a pivotal role in impairing tick attachment, feeding and development [6,63,134].

However, not all hosts are able to mount an effective immune response against ticks. For instance, dogs (*Canis lupus familiaris*) fail to mount an effective immune response against *Rhipicephalus sanguineus* [37,128]. Similarly, the white footed mouse (*Peromyscus leucopus*), a natural host for *I. scapularis* in the north hemisphere, does not develop an effective immune response against this tick specie [10]. In contrast, guinea pig (*Cavia porcellus*), rejects most tick species used in experimental infestation models [5], while, mice (*Mus musculus*) develop protective immunity against *H. longicornis* [91,116].

Finally, certain hosts, such as guinea pigs, cattle (*Bos taurus* and *Bos indicus*) and sheep (*Ovis aries*) can mount an immune response capable of partially impairing ticks during experimentally infestations [10,17,113,115,133]. Taken together, these studies highlight significant variations in the immune response among natural hosts and accidental hosts for specific tick species, emphasizing the importance of host immunity status. This review focuses on local immune responses induced by tick infestation on the host skin, providing insights into the principal immune cells and factors involved in this response based on current knowledge.

Table 1. Immune response investigation at the tick attachment site and cell analysis performed.

Tick species	Host	Cell characterization	Techniques applied	References
Rhipicephalus microplus	Cattle	CD20+ B cell, CD3+ T cell	Biopsy, Histology, IHC, Flow cytometry	[102]
R. microplus	Cattle	WC1+ γδ T cell, B cell, CD4+ T helper	Biopsy, Histology, IHC, Transcriptome	[101]
R. microplus (larvae)	Cattle	Eosinophils, mast cell, mononuclear cells	Biopsy, Histology, RNA extraction, Microarray, qPCR	[97]
R. microplus	Cattle	Eosinophils, macrophages, mast cell, neutrophils, basophils, mononuclear cells, endothelial cell, adipocytes, melanocytes, keratinocytes, dermal dendritic cell	Biopsy, Histology, IHC	[35]
R. microplus	Cattle	CD45+ cells, CD45RO cells, CD3+ T cell, CD8+ T cytotoxic, CD79α B cell, WC1+ γδT cells, MHC II macrophages, DC, B cells, CD25+ activated T cells, CD4+ T, CD25+, CD21+ B cells, granulocytes	Biopsy, Immunofluorescence	[25]

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Tick species	Host	Cell characterization	Techniques applied	References
R. microplus (Larvae)	Cattle	CD3+,CD25+, γδ T, CD4+, CD8+, MHC II cells, granulocytes	Biopsy, Histology, IHC	[24]
R. microplus (Larvae)	Cattle	CD3+, CD4+, CD8+, CD25+, γδ T cells, CD45, CD45RO, neutrophils, eosinophils	Biopsy, Histology, Light microscopy, Immunofluorescence	[26]
R. microplus (Larvae and nymph)	Cattle	Basophils, neutrophils, eosinophils,mast cells, mononuclear, CD3+ T, WC1+T, CD21+ B	Biopsy, Histology, IHC, RNA extraction, Microarray, Tick behavior assay, Tick tissue dissection, Transcriptome	[34]
R. microplus (Larvae)	Cattle	Mast cell, eosinophil, epidermal cells	Biopsy, Histology	[110]
R. microplus (Natural infestation)	Cattle	Eosinophil	Biopsy, Histology	[99]
R. microplus	Cattle	Neutrophil, basophil, eosinophil	Biopsy, Histology, RT-PCR, RNA extraction, Adhesion assay	[22]
R. microplus	Cattle	Mast cell	Biopsy, Histology	[126]
R. microplus (Adult ticks)	Cattle	Mast cell, eosinophil, basophil	Biopsy, Histology	[80]
R. microplus (Natural infestation)	Cattle	Innate immune cells	Biopsy, PCR	[137]
Ixodes scapularis (Nymph)	Balb C/mice	Neutrophil, macrophages, Th1, Th2, regulatory T cell	Biopsy, Histology, PCR, Microarray	[51]
I. scapularis	White footed mice and guinea pig	Neutrophils,macrophages, lymphocytes, plasma cells	Biopsy, Histology, IHC	[10]
I. scapularis	Balb C/mice	Fibroblast, neutrophils, macrophages	Biopsy, Histology, Immunofluorescence	[54]
I. scapularis (Nymph)	Guinea pig and mice	Basophil, mast cell, eosinophil	Biopsy, RNA-sequencing, Histology, ELISA	[70]
Ixodes ricinus	Human	Neutrophil, eosinophil, macrophages, dendritic cells, lymphocytes Th1 and Th2.	Biopsy, Histology, IHC, PCR	[44]
Amblyomma cajennense and *A. dubitatum*	Capybara	Keratinocytes, polymorphonuclear leukocytes with eosinophilic granules, eosinophils, heterophils, mononuclear, mast cell, basophil	Biopsy, Histology, Ultrastructural analysis	[124]
Haemaphysalis longicornis	Mice (Wild and Knockout for T, B cells, FcREI, IL3)	Basophils, CD4+ TRM	Biopsy, Flow cytometry, PCR, Intravital fluorescence microscopy	[91]
H. longicornis	Mice	Basophils	Biopsy, Histology, IHC (mAbmMCP 8)	[129]
H. longicornis	Mice (WT and Knock-out)	Basophil, T cell	Biopsy, Flow cytometry, Cell proliferation assay, Cell culture, Confocal fluorescence microscopy	[115]
Rhipicephalus appendiculatus (Adult ticks)	Rabbit and cattle	Neutrophil, macrophages, eosinophil, basophils, Lymphoblasts, plasmocytes	Biopsy, Histochemistry, Light microscopy, Electron microscopy	[130]
R. appendiculatus and *A. variegatum* (Nymph)	Cattle	Eosinophils, neutrophils	Biopsy, Histology	[71]
Hyalomma anatolicum anatolicum	Cattle	Eosinophils, neutrophils, mast cell, basophils	Biopsy, Histology	[43]
Hyalomma a. anatolicum	Sheep	Neutrophils, macrophage, dendritic cell, lymphocytes CD8+, γδ T cells	Biopsy, IHC	[17]
Ixodes holocyclus	Cattle	Eosinophils, mast cell, neutrophil, basophil	Biopsy, Histology	[8]
I. ricinus	Rabbits	Unknown	Surgical, Histochemical, Histology	[19]

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Tick species	Host	Cell characterization	Techniques applied	References
Rhipicephalus sanguineus	Dog, guinea pig	Eosinophil, neutrophil, eosinophil, basophil, mast cell, mononuclear	Biopsy, Histology, Electron microscopy	[113]
Dermacentor andersoni (Nymph)	Balb C/mice	Inflammatory cells	Biopsy, Histology, PCR, Microarray, histopathology	[54]
Ixodes scapularis	Mice	Inflammatory cells	Microarray, histopathology, PCR, Biopsy	[51]
D. andersoni (Larvae)	Guinea pig	Basophils	Biopsy, Histology	[5]
D. andersoni	Guinea pig	Langerhans cell	Biopsy, Histology	[89]
D. variabilis	3 Mice strains	Mast cell	Biopsy, Histology	[31]
H. longicornis, *Boophilus microplus*, *Boophilus decoloratus* and *R. appendiculatus*	Cattle	Eosinophils, monocytes, neutrophils	Biopsy, Histology	[83]

II. HOST SKIN IMMUNE SYSTEM RESPONSES AGAINST TICKS

Skin serves as the primary physical barrier, constituting the initial defense line against external injuries, including tick mouthpart insertion and represents the first contact between ticks and hosts [51]. Within the skin, resident immune cells such as eosinophils, basophils, mast cells, dendritic cells, and macrophages, actively participate in host defense against tick parasitism [38]. The ability to modulate the host skin immune system is essential for the survival of ticks and the successful completion of their life cycle.

When ticks insert their mouthparts into the skin, they inject saliva containing numerous proteins and other molecules that countermeasures the local host immune system [64]. This successful modulation is essential for the tick's survival and progression through its life cycle. The penetration of tick mouthparts through the skin's epithelial layers triggers a response by the host innate immune system. For instance, human skin infested with *Ixodes ricinus* exhibited higher mRNA levels for chemoattractants responsible for recruiting neutrophils and macrophages, indicating that tick attachment stimulates the host innate immune system [44].

The molecules injected into host skin by each parasite species have variable ability to selectively attract distinct populations of host immune cells to the attachment sites. This phenomenon is widely observed in various ectoparasites. For example, rabbits infested by mosquitoes *Aedes aegypti*, have a different protein expression profile at the skin bite site, compared to non-infested rabbits, highlighting the specific influence of the parasite on the host skin [53]. In mice intradermally injected with sand fly saliva there was an increase in the neutrophil influx at the saliva administration site [46], showing the ability of sand fly saliva alone to initiate cell migration and modulates the host local cellular response in the host skin [126].

Genes related to dendritic cell activation process, maturation and ability to present antigens are upregulated in the pigs parasitized with crusted scabies [14]. Similarly, the skin immune response against the helminth parasite, *Nippostrongylus brasiliensis*, whose earlier development stages migrates through the skin of the host, has also particular characteristics [27]. The larvae L3 of this parasite penetrates the mouse skin induces a local infiltration comprising neutrophils and eosinophils, and induces a Th2 immune response against the parasite [4]. Although transgenic mice constitutively expressing the IL-5 gene are resistant to an initial *N. brasiliensis* infection, some larval stages persist in the subcutaneous part of the skin after 48 hours, suggesting that some larvae did not progress to the next stage and migrate to the lung [68], showcasing the trapping capacity of present cells in the skin [27]. In contrast, at the skin of IL-5 deficient mice (IL-5–/– mice), filaria induces an influx of eosinophils [11]. These studies show the dynamic interplay between parasites and the host skin cellular immune response and reinforce the necessity to do further studies in this study field for effective anti-parasite control.

The host skin immune response to flies that cause myiasis in humans and animals varies according to the parasite species and the physiologic/immunologic status of the host. It manifests through an innate inflammatory reaction, including a cellular and humoral immune response in the skin [92]. Various cell populations in the skin, including sentinel cells, migratory cells, and keratinocytes, play crucial roles in the interaction between parasites and hosts [3,18].

During mouse infestation with *Ixodes scapularis* infected with Powassan virus (POWV), skin biopsies collected at 3 h post-infection exhibited a differential increase in the number and type of cells, mainly neutrophils and macrophages being the first infected cells [54]. However, no differences in histopathological features were observed at 6-, 12- and 24-h post infection. Skin resident memory CD4+ T cells have been also explored in mice infested with various microorganisms vectored by certain ectoparasites [45], including those infested with *Haemaphysalis longicornis* [91]. The number of neutrophils in mouse skin increases 12 h after infestation by *I. scapularis* [51], associated with an upregulation in the expression of genes related to antimicrobial responses, reactive oxygen species, wound healing, chemokines and cytokines consistent with the influx of these cell [51].

Cattle primarily infested with the tick *Hyalomma anatolicum anatolicum* shows an increased number of neutrophils at the tick bite site, followed by a fast increase in the mononuclear cells, in contrary with the prominent and higher increased number of basophils, neutrophils, mononuclear in a tertiary infestation in 24, 72 and 144 h post infestation [43]. These findings correlate with the manifested immunological resistance at the tick bite site and the impaired tick biological parameters.

Concerning ticks and cattle interaction investigated the local immune response of bovines infested with *Rhipicephalus microplus* larvae and suggested that CD3+, CD25+ and γδT cells play a role in skin immune response against tick larvae as these cells subpopulations are augmented at the tick attachment site [24]. However, the specific role of these cells remains to be elucidated. Furthermore, the number of eosinophils, neutrophils and macrophages increase at the tick attachment site at the beginning of bovine infestation by *R. microplus* [35]. Interestingly, host-immune cells such as neutrophils were found inside the tick body during cattle infestation with *R. microplus* larvae [24]. Apparently, these cells are taken from the cavity lesion during the meal. Indigenous East African Zebu infested with *Rhipicephalus appendiculatus* and *Amblyomma variegatum* presents an infiltrate in the skin composed by a higher number of eosinophils and neutrophils relative to other cell types at the tick attachment site [24]. Comparing Zebu (*Bos taurus indicus*) with lower tick-resistant cattle (*Bos taurus taurus*), the number of neutrophils decreases in cattle infested with *R. appendiculatus* nymphs [71]. On the other hand, no differences were found in the number of neutrophils, when comparing the skins of resistant and susceptible breeds infested with adult *R. microplus* ticks [22]. This study corroborates an earlier study showing no difference in the number of neutrophils in the skin of Zebu infested with *A. variegatum* compared to Holstein Friesian, a less tick-resistant breed [72]. It was hypothesized that this type of cell increases at the tick attachment site independently of the tick stage (larvae, nymph or adult or the host susceptibility to ticks [22,34]. Still, the precise effect of each tick species on the composition and activity of the infiltrated cells at the tick bite site remains to be elucidated.

III. TICK-HOST INTERFACE

1. Tick interaction with the host skin immune system

Ticks have evolved interacting with their hosts [76], and both tick and host contribute to define the features of the attachment site interface [23]. On the host skin, not only does injury from tick mouthparts induce an inflammatory process, but also salivary molecules injected by ticks cause epithelium stratification, hyperemia, edema, immune-inflammatory cell migration and the pathogen transmission [50]. Tick saliva contains proteins able to modulate host skin defense mechanisms, including inflammation, coagulation, wound repairing and adaptive immune response [38,118]. Soon after tick attachment, most ticks of the family Ixodidae secretes variable amounts of salivary proteins to form the cement cone, which serves as an adhesive for tick fixation in the host skin [73,94]. This cement, formed mainly by glycine rich proteins (GRPs), exhibits antimicrobial and adhesive properties and acts to seal the lesion during feeding. It facilitates fixation, pathogens transmission, and protection from mammalian host immune and inflammatory responses [94].

The cement cone plays a role as a substance that anchors hard tick mouthparts to the host skin, sealing the feeding lesion and facilitating the tick feeding [16]. Cement cones have 2 layers, composed of proteins that tick secretes into the saliva at various times during attachment [56]. GRPs have been identified in many Ixodidae tick such as *Ixodes scapularis*, *Amblyomma cajennense*, *A. americanum*, *A. maculatum*, *Rhipicephalus microplus*, *R. appendiculatus*, and *R. pulchelus* [56,73,86]. Additionally, glycine-rich proteins have been suggested to be involved in antimicrobial mechanisms, avoiding the host immune response as well as tick borne-disease transmission [38,49,56]. Nevertheless, additional functions of the majority of cement proteins remains still secret [86]. A cement elementome study show ticks express a plethora of molecules in cement and its composition varies among different species and life stages [94]. Some GRPs genes were silenced by RNA interference (RNAi), affecting the cement formation, demonstrated that they are important in the feeding process [56]. This data open the possibility that GRPs and other proteins can be targets for tick control.

Another suggested function for the cement cone is protection against the immune system. It forms a physical barrier to prevent tick mouthparts from contacting host immune molecules [15,16]. Because of this immune system related functions, these proteins have been exploited as possible vaccinal antigens with promising results [16,73,122,140].

Tick salivary proteins also induce changes in the host gene expression profile at tick attachment site, interfering in important pathways related to skin defense. Conversely, host local immune responses affect the genes encoding tick-secreted proteins [39,41,120]. Differentially expressed genes related to inflammation were upregulated in the skin of susceptible cattle during larvae and nymph infestation with *R. microplus* [34]. Tick parasitism, consequently, alters dermal architecture and migration of inflammatory cells such as granulocytes, monocytes at the tick attachment site [10]. Phagocytosis-mediated by neutrophils and macrophages serves as the frontline defense for the clearance of foreign substances and necrotic debris in skin [10,24,26]. The contribution of these cells at molecular level is not yet well elucidated and needs an accurate investigation at the host skin interface in order to determine the factors underlying the host defense mechanism against parasites. Some reviews highlight the cellular count at the host skin and point out many gaps that deserve more studies to have a clear picture of the skin immune response against ticks [62,101,117]. For example, found that in rabbit infested with *Haemaphysalis longicornis* larvae, nymph and adults, a subunit of the NADPH oxidase gene is upregulated suggesting that this system is active at each stage of tick life cycle, and that host antagonize the action of tick foreign active substances from tick saliva [139]. Reactive oxygen species (ROS) produced by phagocytes in the skin shows that phagocytosis is activated to eliminate exogenous substances in the host skin and tissue debris present at tick bite site [24,139].

Ticks have evolved mechanisms to impair the antigen presenting process during the attachment phase [38]. In this sense, calreticulin and calnexin may participate directly or indirectly in the process of phagocytosis, as these proteins play an important role in the antigen presentation process [40]. Indeed, these proteins are upregulated in the skin of rabbits infested with the tick *H. longicornis* [139]. Moreover, the increased expression of lectin in the skin of tick-infested rabbits suggests that this protein should be used as a marker of the inhibition of the skin immune response. Future works should also include the entire tick parasitic life cycle to understand fully the host skin anti-tick immune system mechanisms.

2. Cellular migration and inflammation

Inflammation is the frontline defense against tick feeding [119] triggering the migration of immune cells to the site of tick bite [51,91]. It is suggested that early tick feeding initiates an inflammatory response which intensifies as feeding progresses [51]. The type of immune cell migrating to the tick bite site is determined by the differential expression of adhesion molecules, influenced by the host genetic composition and the level of tick infestation [22]. In this sense, an upregulation of genes related to adhesion molecules, such as ICAM3 (Intercellular Adhesion Molecule 3) was observed in the skin of pig infested with mites *Sarcoptes scabiei*, while molecules associated with immune cell recruitment and trafficking were downregulated [14]. For example, selectins and integrins adhesion molecules present on endothelial cells and leukocyte surfaces, are modulated by ticks in order to impair leukocyte migration [22,69]. In mice infested with *I. scapularis*, an upregulation of NFkB family genes, including Nfkbia, Ikbz, and Nfkbiz was observed, associated with pro-inflammatory cytokines and

chemokines crucial for the early (6 h) immune response in the tick-host interaction [52]. Moreover, in the same study, at 12 h after infestation, genes related inflammation and chemotaxis had increasing in the expression [52]. The chemokines are potent activators and chemoattractants for leukocytes and play a crucial role at the sites of inflammation. The upregulation of genes related to IL-1b, IL-6, and C1qb (molecules known to interact with the chemokine to maintain and amplify the chemotactic response, and affecting the recruitment of neutrophils and monocytes to the bite site potentially impacts tick engorgement and tick-borne viral transmission [51].

Skin of cattle with different phenotypes infested with *Rhipicephalus microplus* showed differential expression of mRNA of ICAM-1 (Intercellular Adhesion Molecule 1), VCAM-1 (Vascular Cell Adhesion Molecule 1), LFA-1 (Lymphocyte Function-Associated Antigen 1), P-selectin and E-selectin in the skin [22]. Exception was observed for LFA-1, which was upregulated only in susceptible *Bos taurus indicus* animals during low or high infestation compared with resistant animals [22]. More recently, an analysis of bovine skin transcriptome before and after repeated *Rhipicephalus australis* challenge revealed an increase in interferon type 1-stimulated genes expression in the skin of tick-susceptible animals [78]. The authors suggested that disrupted healing of wounds and prolonged inflammatory skin conditions might contribute to the host's vulnerability to ticks.

Inflammation, wound healing are interconnected processes that can be influenced by leukocyte migration and the expression of adhesion molecules. *In vitro* studies reveal that tick saliva molecules bind and inhibit various proteases involved in host homeostatic mechanisms, including chymotrypsin, cathepsin G, factor Xa, trypsin, chymase, plasmin [119], as well as molecules binding to adhesion molecules [22]. Ticks secrete proteases inhibitory proteins that participate in host homeostatic systems and impair leukocyte migration to the bite site [69].

In the following section, the main skin immune cell populations in tick-host interaction are considered in more detail.

IV. SKIN IMMUNE CELLS

1. Dendritic cells

Dendritic cells (DCs) are the major antigen presenting cells in the skin immune system [60]. During blood feeding, parasites inject molecules, potentially antigens, which are taken up by DC initiating an adaptive immune response [53]. The role of DCs is crucial in defining the outcome of the immune response in the interaction between parasites and their hosts. This outcome depends on the modulation induced by specific parasite salivary molecules injected, leading to either high or low host susceptibility or resistance to the infestation [107,109]. In the case of *Aedes aegypti*, the number of mice DCs at the attachment site decreases 24 h after the mosquito bite, indicating a role for DCs in mosquito parasitism [53]. Pigs infested with *Sarcoptes scabiei* show a downregulation of genes related to DC subpopulation CD1B maturation and antigen presentation process [14], while, DC CD207 has an upregulation of genes related to antigen presentation [14].

Several studies have demonstrated that molecules in tick saliva modulate DC functions in various tick species [69]. Salivary molecules secreted by tick can impair DCs maturation and antigen-presenting capability through the blockade of DC pattern recognition receptors (PRRs) [69]. Genes related to DC maturation and antigen-presenting process were downregulated in the skin of mice infested with *Ixodes scapularis* at 3, 6 and 12 h of infestation [52]. Interestingly, the expression of C-type lectin genes increased in the skin of mice infested with *Dermacentor andersonii* [51], suggesting a role in phagocytosis and antigen-processing mechanisms at the skin, where tick saliva molecules may engage the C-type lectin receptor in the DC [51]. However, the exact function of this type of receptor in tick-host interaction remains undefined.

Langerhans cells, a subset of epidermal skin DCs, interacts with tick saliva antigens in guinea pig infested with *D. andersonii* larvae [7]. This study showed salivary gland antigens associated with the suprabasal DC layer in the epidermis trapping these antigens during secondary, but not primary infestation, suggesting a critical role of specific DC subpopulations at the skin-tick attachment site [7]. A *Rhipicephalus haemaphysaloides* serpin inhibits DC co-stimulators B7 (CD80 and CD86) influencing the expression of inflammatory cytokines and impairing the tick feeding process [135]. This study reinforces the importance of studying DC subsets induced by tick antigens and tick secreted small molecules. Guinea pigs infested with *D. andersoni* had an increase the number of Langerhans cells at the tick attachment site during infestations, reinforcing the critical role of specific DC subpopula-

tions at the skin tick attachment site [89]. The Inhibition of DC functions by tick saliva also reveals the importance of DC in the skin at the tick-host interface [36,106,109]. For example, mRNA levels of the dendritic cell marker CD11c and other mRNA related to innate immune response were significantly increased in skin of humans infested with *Ixodes ricinus* compared to control skin [44]. Despite extensive research on tick and host cellular DC, the contribution of each DC skin subpopulation to the tick-host interaction is still unknown [106].

2. Basophils

Basophil is a granulocyte related to the inflammatory process during skin damage caused by parasites. Basophils are detected in infiltration at the skin lesion in humans infested with mites *Sarcoptes scabiei*, and bed bug *Cimex lectularius* [59]. Evidence suggests their participation in a protective immune response against ectoparasites [62]. Moreover, their numbers are higher in the skin of resistant cattle breeds compared to susceptible phenotypes infested with *Rhipicephalus microplus* [22]. Similarly, Nguni cattle (an African indigenous cattle breed) had a higher basophils count compared to Bonsmara (a 5/8 Afrikaner, 3/16 Shorthorn and 3/16 Hereford) cattle infested with *R. microplus* [80]. In mice, basophils are recruited to the tick attachment site through the interleukin 3 produced by peripheral and resident memory CD4+ T cells in skin [91]. Ablation of basophil in guinea pig demonstrated that these cells play an essential role in acquired host immunity against ticks [5]. Histamine released by basophil is described as an essential mediator of acquired tick resistance and plays a fundamental role in tick rejection, acting on skin epithelium, causing cell hyperplasia, itching and grooming [19,116]. Although basophils are scarce in circulating blood, their number increases in the skin of the cattle (for example, in the crossbred Holstein) infested with *R. microplus* [35]. Since the seminal studies of infiltration of basophils and other granulocyte cells has been related to acquired tick resistance (ATR) and subsequent research has highlighted their role in ATR development at the skin [62,91,121,130]. Depletion of basophil was shown to decrease ATR in guinea pigs infested with *Amblyomma americanum* [20]. The same author demonstrated that basophils are the first cell to infiltrate cattle skin, with variations observed depending on tick species. Basophils were identified as the primary infiltrating cells, even in cattle previously infested with *Ixodes holocyclus* adult ticks [8]. Despite being considered a hallmark of ATR, the role of basophils in cattle, rabbits and goats infested by ticks remains to be investigated [63,61]. Basophils are the base for current cellular and molecular mechanisms proposed for ATR developed in animal laboratory experiments [136].

Mice infested with *Haemaphysalis longicornis* recruit basophils to the tick bite site during second infestation [130]. Another report demonstrated a basophil infiltration at the tick feeding site during *H. longicornis* reinfestation in mice [82]. Although sometimes it is difficult to detect basophils due to their characteristic containing a few granules, they were effectively observed by infiltrating and progressively increasing in concentration in basophil cell-sufficient/ mast cell deficient mice after 3 *Dermatocentor variabilis* infestations [130]. Basophils depletion in mice completely impairs ATR, without affecting the number of other cells in the skin of mice infested with *H. longicornis* [130]. Basophil activation was observed in human skin infested with *Ixodes persulcatus* [59]. Furthermore, histamine released by basophil, and not by other granulocyte cells, is essential for ATR and consequently for tick bite impairment in mice [116]. Skin injected with histamine displayed more epidermal hyperplasia, demonstrating that histamine released by basophils is crucial for the host immunological local resistance capable of impairing ticks [116].

Using a murine model, it was proposed a mechanism of basophils in the defense against tick infestation [61,63]. In this model, during the first tick infestation, DC takes up tick saliva antigens and moves to draining lymph nodes presenting antigens to the CD4+ T cells. Also, there is IL-4 secretion by these cells. IL-4 derived from T cells stimulates B cells to produce IgE to tick antigens, which in peripheral blood bind to basophils [136]. In turn, some CD4+ T cells migrate from the lymph node to the skin. At the second infestation, CD4+ T cells are reactivated by tick antigens and promote the liberation of IL-3, which recruits basophils to the tick bite site, progressively increasing in concentration number and releasing histamine that acts on keratinocytes, resulting in the epithelial hyperplasia that causes tick impairment [61,63,91,136].

3. Mast cells

Mast cells are also vital immune cells involved in host skin response against tick parasitism [101,116].

Ticks secrete lipocalins which act as kratagonists, binding the histamine released by mast cells. Although this action has not been studied at the onset of parasite host relationship, the importance of mast cells for ATR during parasitism varies according to the tick and host species [31,112]. For example, FcεRI signaling pathway related to mast cell is upregulated at the skin bite site of guinea pig infested with *Ixodes scapularis* nymphs [70], suggesting their participation in mediated hypersensitivity process [91]. This same pathway is not activated in mice subjected to the same infestation protocol, suggesting specific host immunological responses regarding the FcεRI signaling pathways. The differences in such pathways may be influenced if the host is natural or non-natural and the tick species. Interestingly, mast cell-deficient mice failed to exhibit ATR, but when mast cells were transferred to the same mice, ATR was recovered [82], suggesting mast cells are essential for the ATR in some tick-mouse infestation models. Studies developed by [112], demonstrated by histological skin analysis that mast cells have a relevant role in the mice ATR against *Dermatocentor variabilis* infested in mast cell-deficient W/Wv mouse. Finally, mast cell degranulation was increased in the skin in the first 12 h after infestation with *I. scapularis* in mice [51].

Although many studies, the mast cell specific mechanisms in host protection against ticks at the host skin attachment site remains unknown.

4. Eosinophils

Eosinophils, abundant during allergic inflammation and helminth infections, also play role in ectoparasite infestations, including tick infestations [13,53]. During inflammation, eosinophils are recruited to host skin, releasing molecules harmful to ectoparasites [58]. A comprehensive understanding of eosinophil involvement in the host skin immune response against ectoparasites could enhance the understanding of the host parasite relation, as proposed by [35].

Eosinophil influx increases during *Sarcoptes scabiei* infection in humans [13]. Histopathological studies of human scabietic infection showed an increase in dermal eosinophilic infiltrate [34]. Similarly, crusted scabies patients exhibited elevated eosinophil influx in the skin [132]. Eosinophils also contribute to anti-helminthic defense reviewed by Huang [58]. *Psoroptes ovis* infestations in sheep, cattle, and red fox (*Vulpes vulpes*) result in lesions with a predominant inflammatory infiltrate composed of eosinophils [74,124].

Eosinophil roles have been studied in cattle, aiming to discern differences in eosinophils influx at the tick attachment site during different tick life stages [35,39]. Observations revealed a greater influx of eosinophils in the skin of naïve calves of susceptible cattle breeds in comparison with calves previously infested with ticks [97]. Distinct patterns were noted in eosinophil influx in cattle skin infested with the larvae of different tick species and life stage [39]. The susceptible cattle exhibited a significantly higher eosinophil count compared to resistant animals, even those previously infested [35,101]. Interestingly, this count decreases during infestation with adult *Rhipicephalus microplus* ticks [22]. In field conditions, where animals are infested with multiple tick species or experimentally infested with *Hyalomma anatolicum* and *R. appendiculatus* adult ticks, there is a progressively increasing eosinophil numbers degranulation. Indeed, seminal works established a positive correlation between the increase in eosinophil numbers and the number of infestations [43,131]. In crossbred Holstein cattle infested with *R. microplus* [35] and in the skin of guinea pig infested with *Ixodes scapularis* [70], eosinophils are prominent in the dermal site. While mouse infested with *I. scapularis*, eosinophil numbers increase during the first 3 h after infestation and remains augmented at the skin attachment site during course of experimental infestation [51]. In cattle infested with *R. microplus*, eosinophils are prominent in the dermis, constituting the majority of cells infiltrating the profound dermis [35]. During tick infestation in cattle, as larvae molt to nymph, eosinophil influx increases maintaining this pattern through the adult life stage [39]. Studies with cattle infested with adult tick *R. microplus* also showed an increased number of eosinophils [22]. Noteworthy differences in eosinophil counts were observed in 2 tick-resistant cattle breeds (Bonsmara and Nguni). The influx of eosinophils in the skin infested with *R. microplus* was higher in Nguni heifers than in Bonsmara heifers [80]. Eosinophils count also increase at the attachment site in indigenous East African Zebu cattle infested with *Amblyomma variegatum* and *Rhipicephalus appendiculatus* compared to susceptible Holstein Friesian cattle [72]. Furthermore, the influx of eosinophil increases at the tick *Hyalomma anatolicum anatolicum* bite site in cattle after 24, 72 and 144 h in

the tertiary infestation, even after the animal manifests the resistant phenotype [43]. Eosinophils may play a crucial role in the establishment of infection and the transmission of the pathogens transmitted by ticks [108]. Eosinophil in the host immune response against ticks requires further investigation of the molecular mechanisms involved in such cellular response.

5. γδT Cells

γδ T cells are a subset of T cells that express a T cell receptor (TCR) composed of gamma and delta chains, rather than the alpha and beta chains found in conventional T cells [57]. They play roles in immune surveillance and responses distinct from αβ T cells [57].

Experiments investigating the role of γδT cells in the immune response against ticks found their enrichment at the parasite tick attachment site, proposing speculations about their relevance in immunity against ticks [22,24,26,98]. It was reported higher levels of γδT cells in the skin of resistant animals compared to susceptible animals [24]. A similar pattern of γδ T cells was also observed in the analysis of skin from resistant and susceptible cattle [39]. Moreover, it was suggested that γδT cells play a regulatory role in bovines [55]. However, the role of γδT cells in host immune defense against ticks needs to be further investigated.

V. ANIMAL MODEL TO STUDY SKIN IMMUNE RESPONSE AGAINST TICK

Demonstrations of immune responses and impaired tick feeding and pathogen transmission in non-natural host subjected to repeated tick infestation underscore the importance of alternative models for comprehensive tick physiology analysis and tick control method development [5,87,88,134]. Animal models like rabbits, guinea pigs, mice, and hamsters have been utilized to study host immunity against various tick species [5,10,91,103,139]. However, the complexity of tick-host interactions requires improved precision, as physiological mechanisms differ between ticks in natural and non-natural host interactions. Current tick management faces challenges due to tick species' promiscuity, enabling adaptation to alternative hosts when preferred hosts are unavailable [120].

It has been demonstrated that guinea pigs, rabbits and cattle produce an effective immune response against various tick species [7,10,24,70]. Cattle acquire partial immunological resistance during the initial infestation, primarily directed against larvae [24]. This acquired immunity results in reduced molting larvae number, decreased engorged female weight or even death of engorged ticks in primary or subsequent infestations [70,121]. Differences in local immune responses to tick infestation exist between *Bos taurus taurus* and *Bos taurus indicus* [39].

In fact, Holstein *B. t. taurus* and Nelore *B. t. indicus* infested with tick larvae and nymph, exhibit variations in skin cell profiles [22,24]. Also, it was shown that adhesion molecules at the skin of infected calves significantly differ between Nelore and Holstein breeds [22]. Also, it was demonstrated that Brahman cattle have a delayed hypersensitivity compared to Holstein and presents more neutrophils at the tick attachment site [24]. These findings highlight the variability of local immune response to tick infestation varies according to cattle breed.

The development of acquired immunity depends on the interaction between ticks and its hosts [66,119]. The ability to secrete some particular molecules into the host and the host response to them are influenced by immune heritable characteristics [66,70,103]. It is clear, the host also modulates the secretion of salivary proteins, as observed in the *I. scapularis*, *A. americanum* and *R. microplus* stimulated by contacting, before the feeding process, in different host species as rabbits, cattle, humans, dog and cattle, respectively [66,100,105,120]. Tick saliva secretion depends on the host genetic composition and ticks secrete different molecules according to specific developmental stages [41,66,105,118,120]. These factors lead to a diversity of outcomes regarding immune reactions cellular profile at the tick-host interface. For example, *Ixodes scapularis* do not induce a protective immune response in its natural host, the white footed mice, despite the strong inflammatory reaction at the tick bite site [10]. In contrast, guinea pig mounts a robust immune response within hours of tick attachment (around 6 h) [10], resulting in dermal inflammation capable to reject *I. scapularis*. Guinea pig also rejects *Rhipicephalus sanguineus* [12]. Rabbits develop a strong tick rejection impairing ticks from completing their parasitic cycle [121]. The challenge is to translate findings from these models to natural and accidental hosts, providing insights for improved tick control. While these models offer experimental convenience and cost-effectiveness, results must be validated in the natural host of each tick species.

VI. CONCLUSIONS

The local skin immune response to tick and other ectoparasite infestations is intricately influenced by the microenvironment created by parasite attachment components and secreted proteins, attracting and engaging local immune cells. Host immune status further contributes to this dynamic.

This review underscored the vital role of innate immune cells at the tick-host interface. However, a comprehensive knowledge about skin immune responses to ticks and other ectoparasites still far from be achieved and additional studies are imperative to address the gaps. For example, an exploration of B-cell responses and heterogeneity, along with an in-depth investigation into the complete Th2 immune response mechanism, will contribute crucial insights for refining existing tick control methods.

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