

AN INSIGHT INTO THE HISTOPATHOLOGY CAUSED BY THE TICK *Rhipicephalus sanguineus*
(Acarina: Ixodidae) IN THE SKIN OF PREVIOUSLY INFESTED, VACCINATED OR
TICK-BITE NAIVE DOGS, GUINEA PIGS AND HAMSTERS*

MOMENTO HISTOPATOLÓGICO NA PELE DE CÃES, HAMSTERS E COBAIAS SOFREDO INFESTAÇÃO
EXPERIMENTAL PELO CARRAPATO *Rhipicephalus sanguineus* PELA PRIMEIRA VEZ OU APÓS
VACINAÇÕES OU INFESTAÇÕES PRÉVIAS

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SUMMARY

Dogs', guinea pigs' and hamsters' local skin reaction to the attachment and feeding of the tick *Rhipicephalus sanguineus* were analysed under light microscopy. The hosts were either tick-bite naive, previously infested or vaccinated with crude unfed adult tick extract. Biopsies were taken at the end of each infestation. Changes common to every host, regardless of experimental group, included presence of tick or its mouthparts embedded in a cone of cementum at the surface of the skin, epidermal hyperplasia, hyperkeratosis and acanthosis, edema and copious inflammatory cell infiltration in the dermis, underneath the tick attachment site. Dogs in all experimental groups reacted with an almost exclusive PMN neutrophil accumulation, while guinea pigs showed a predominantly mononuclear cell infiltration in every experimental group. Hamsters suffering first infestations had a mainly neutrophilic infiltration, showed a predominantly mononuclear cell infiltration in response to multiple infestations, and when previously vaccinated this host had a predominantly eosinophilic infiltration. The persistence of PMN neutrophils in dogs suggest a control of the local immune inflammatory response by the tick. The predominantly eosinophilic infiltration in previously vaccinated hamsters might indicate that different immune mechanisms were triggered by infestation and vaccination.

UNITERMS: Histopathology; *Rhipicephalus sanguineus*; Dogs; Hamsters; Guinea pigs

INTRODUCTION

Although most studies have shown that there is naturally acquired resistance to ticks in many hosts (HEWETSON¹⁹, 1971; WIKEL et al.²⁶, 1978; GEORGE et al.¹⁷, 1985), some have demonstrated a lack of resistance in hosts to ticks, even after repeated feeding (CHABAUD¹², 1950; RANDOLPH²³, 1979).

General histopathologic aspects associated with tick attachment to vertebrate hosts include epidermal hyperplasia and vesiculation, vasodilation, hemorrhage, edema and intense cellular infiltration of the dermis. The cutaneous cellular response at tick feeding sites in tick-bite naive animals is characterized primarily by PMN neutrophil infiltration of the skin beneath the attachment site (BROWN⁸, 1988). Interestingly, in most studies, hosts showing resistance to ticks (guinea pigs and bovines) displayed a marked cutaneous basophil accumulation during subsequent infestations (ALLEN et al.², 1977; ASKENASE et al.³, 1982; BROWN et al.¹⁰, 1984). This cutaneous basophilic infiltration was

correlated with resistance (BROWN et al.¹¹, 1982). On the other hand, THEIS; BUDWISER²⁴ (1974) observed that cellular response of dogs to the tick *Rhipicephalus sanguineus* was characterized by an intense cutaneous PMN neutrophil infiltration at any time after tick attachment, even after repeated infestations. Intriguingly, dogs seem unable to develop resistance against this tick species (CHABAUD¹², 1950; GARIN; GRABAREV¹⁶, 1972; SZABÓ et al.^{**}) whereas hamsters and mainly guinea pigs develop a strong resistance to the same tick species which follows repeated infestations (SZABÓ, et al.^{*}) or vaccinations with crude unfed adult ticks (BECHARA et al.⁴, 1994).

The aim of the present work was therefore to compare histopathologic aspects of the *Rhipicephalus sanguineus* tick attachment sites in tick-bite naive, previously tick infested or vaccinated dogs, guinea pigs and hamsters at the end of an experimental infestation.

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MATERIAL AND METHOD

Ticks: A *Rhipicephalus sanguineus* tick colony was set up in the laboratory, in order to supply the experiments with unfed adult ticks. Initially, engorged females were collected from dogs of the Veterinary College Hospital in Jaboticabal, Brazil. Once identified, they were kept under conditions of constant temperature and relative humidity (29°C and 80% respectively). Continuous tick supply was achieved by feeding each instar on tick-bite naive guinea pigs.

Hosts: Three animal species were used in these experiments: mongrel dogs (*Canis familiaris*); hamsters (*Mesocricetus auratus*) and guinea pigs (*Cavia cutleri*). Dogs were used as the natural host of *Rhipicephalus sanguineus* ticks, and the other two species as they are commonly used laboratory hosts.

Every animal was initially tick-bite naive and for this purpose we bred, dogs, hamsters and guinea pigs in tick-free conditions. Guinea pigs weighed approximately 500 g and hamsters about 120 g at the beginning of the experiments. Dogs of approximately six kilograms and 5 months of age were used. Water and food were given "ad libitum".

Repeated infestations: Each animal species was subjected to 3 successive infestations using unfed adult *Rhipicephalus sanguineus* ticks. Infestations, one month apart, consisted of 4 female and 5 male ticks on each rodent, and 25 females and 30 males on each dog. In the case of rodent trials ticks were placed inside a feeding chamber consisting of a plastic tube having a diameter of 2.5 cm and a height of 3 cm. The chamber had been glued to the shaved back of the hosts on the day prior to addition of the ticks. Chambers placed on dogs were 5.0 cm in diameter and 3 cm high. Neck collars were also used to prevent grooming. In order to avoid the escape of ticks during the experiments, hosts were kept in cages placed in trays surrounded by a gutter filled with water and oil.

Tick extract: About 500 unfed adult *Rhipicephalus sanguineus* were obtained from the colony, killed by immersion in liquid nitrogen, homogenized with a ground glass homogenizer in PBS (Phosphate Buffered Saline) and then sonicated for 10 seconds 3 times and once for 60 seconds (intensity of 20 mHz). This extract was centrifuged at 4°C for one hour at 12,000 g, the supernatant filtered through Millex-GV (Millipore) filter with pores of 0.22 micrometers and stored at -40°C until used. The protein concentration determined according to LOWRY et al.²¹ (1951) was of 2.5 mg/ml.

Vaccination of hosts: The immunization procedure was similar in all three hosts. Hamsters, dogs and guinea pigs were inoculated subcutaneously with the unfed adult extract (UAE). Inoculations were given three times, at 15-day intervals. Each hamster and guinea pig received 125 mcg of extract plus 50 mcg of saponin (Quil A, Superfos Biosector A/S, Denmark) as adjuvant and diluted in PBS

to a final volume of 1.0 ml per dose. Dogs were treated the same, but the protein content was doubled.

Challenge infestation: Fifteen days after the last inoculation, each vaccinated host was submitted to a challenge infestation with unfed adult ticks. Non-infested controls, tick-bite naive and unvaccinated animals were also included. The infestations were performed as described above.

Biopsies: Biopsies from tick attachment sites were taken from 3 dogs, 5 hamsters, and 5 guinea pigs that had been given repeated experimental tick infestations and 2 dogs, 3 hamsters and 3 guinea pigs that had been vaccinated with tick extracts and challenged later with an experimental tick infestation. Biopsies of about 1 cm³ were taken at the tick attachment sites at the end of infestations 1, 3 and the challenge infestation in each host. Biopsies included the last engorging female, just prior to its detachment and males which remained attached. Skin samples were immediately immersed in Bouin-Dubocsq fixative (BEHMER et al.⁵, 1976). Normal, unparasitized skin samples were also taken to make comparisons.

Histotechnological processing: Skin samples were kept for 24 hours in the fixative, embedded in paraffin and processed according to routine histological techniques. Each biopsy was cut longitudinally through its center, sectioned at a thickness of 7 micrometers, and stained. Hematoxylin-eosin, Toluidine blue and May Grünwald-Giemsa were used to distinguish as many cell types and structures as possible.

Sections were observed under light microscopy. No cell counts were performed once observed lesions had different ages, as the feeding period of ticks from each sample varied a lot, due to increasing resistance of the hosts and differences among hosts' reactions.

RESULTS

General features of attachment site histology

Gross local changes to tick attachment were minimal in dogs and hamsters during all infestations. Slight hyperemia and occasional skin thickening were seen when many ticks fed closely to each other. Guinea pigs, on the other hand, displayed a very strong local reaction to ticks, mainly during infestations 2 and 3, and to a lesser extent during the challenge infestation following vaccination. Intense hyperemia, swelling, fluid exudation and even necrosis could be seen. Guinea pigs' behaviour during these infestations suggested that they were being severely irritated by the ticks.

As for microscopic features, ticks, or parts of them could be seen in every experimental situation with the hypostome embedded in the cone cementum (Fig. 1). The attachment was always very superficial being restricted to the initial

layers of the dermis. Cementum could be seen as an eosinophilic homogeneous mass over the epidermis, which widened progressively toward the hypostome (Fig. 2). This substance did not extend deeply into the dermis, usually being confined to the surface of the epidermis or extending under the stratum corneum. The epidermis surrounding and under the cementum cone was always thickened mainly due to hyperplasia, hyperkeratosis and acanthosis. Just under the tick's attachment site and the cement substance, a vesicle in the tissues could be seen filled with dead cells and debris; this is known as feeding cavity (Fig. 2). The thickening of the dermis was caused mainly by an intense cellular infiltration, whose characteristics varied according to the host species and type of infestation involved and by edema, as shown by the dissociation of fibers from the connective tissues. Newly formed vessels and fibrosis in many sections indicated the onset of a healing process in the dermis. Basophils were not seen in any host in any experimental condition. Mast cells tended to be localized near dermal vessels. Mononuclear cells included macrophages, lymphocytes and fibroblasts as it was difficult to distinguish these cell types under light microscopy.

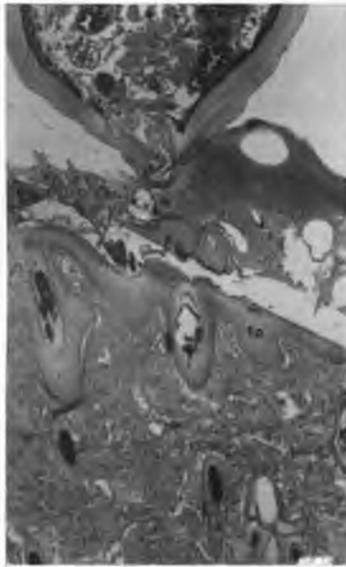


FIGURE 1

Photomicrograph of the attachment site of the tick *Rhipicephalus sanguineus* (Rs) on a dog. Arrow = cement substance. Ep = epidermis. May Grünwald-Giemsa (x66).

Reactions in hamsters

In hamsters, apart from the general features just described, the first infestation was characterized by dermal infiltration with predominance of PMN neutrophils, some eosinophils and some degranulating and whole mast cells. After the third infestation, the infiltration was dominated by mononuclear cells and some eosinophils. Hemorrhage, as suggested by local hemosiderosis, was also noted. Mast cells from deeper portions of the dermis were intact, while some of those close to the tick's attachment site showed some degranulation.



FIGURE 2

Photomicrograph of a guinea pig's skin during third infestation with *Rhipicephalus sanguineus* ticks. Ce = cement cone. Fc = feeding cavity. Ep = epidermis. Hematoxylin-Eosin (x66).

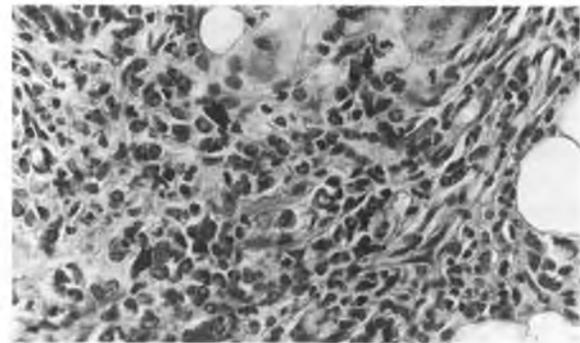


FIGURE 3

Photomicrograph of a hamster's skin during a challenge infestation with *Rhipicephalus sanguineus* ticks. Arrows = eosinophils in the superficial dermis. Hematoxylin-Eosin (x660).

Focuses of necrosis in dermal muscles were characterized by absence of transversal striations and by decreased eosinophilic staining. After challenge infestations in vaccinated animals, most mast cells seemed to be intact, and the dermal infiltration was predominantly eosinophilic (Fig. 3).

Mononuclear cells and rare neutrophils were also seen. In this host the infiltrating cells extended into the deep dermis and into the hypodermis.

Reactions in dogs

The histopathologic patterns seen in all 3 types of infestations on dogs were very similar one to the other. The main element was a cellular infiltration dominated overwhelmingly by PMN neutrophils which amounted to 100% of the cells close to the attachment site of the tick (Fig. 4). The cellular infiltration,

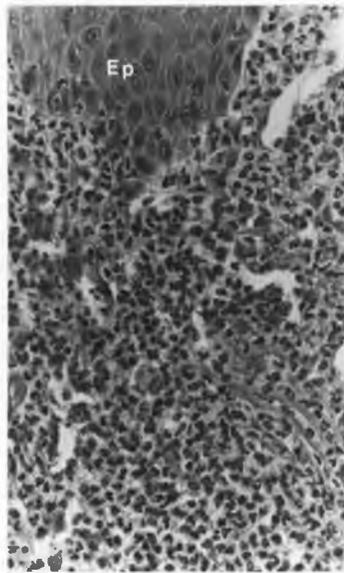


FIGURE 4

Photomicrograph of a dog's skin just under the attachment site of a *Rhipicephalus sanguineus* tick during first infestation. Ep = epidermis. Hexatoxylin-Eosin (x660).

although very intense underneath the cementum, did not reach deeper layers of the dermis nor did it spread laterally. Degranulated mast cells were not seen in any of the dogs.

Reactions in guinea pigs

In guinea pigs, there were no predominantly neutrophilic or eosinophilic infiltrations. Mononuclear cells dominated the infiltrations in all infestations. Significant numbers of neutrophils were seen only during first infestation. The inflammatory cell infiltration in guinea pigs during infestation 3 and challenge after vaccination was very extensive reaching deeply the hypodermis and even to the subcuticular muscle layer (Fig. 2). Acantholysis and small pustules were observed beside the cementum in sections from the third infestation of guinea pigs. In this host, thickening of the epidermis was particularly evident at that time, mainly due to hyperkeratosis.

DISCUSSION

It is clear that this histopathological analysis is partial because biopsies were only taken at the end of each infestation. Female tick detachment times and feeding periods varied considerably among hosts and types of infestations, probably due to the onset of resistance, and consequently biopsies represented differing conditions and observed lesions had different ages. Moreover, considering that the end of each infestation occurred after at least 5 days of attachment and feeding, elements of the healing process were already seen. These conditions rendered impossible the performance of comparative cell counting.

However, many interesting observations were made during the histopathological analysis.

General histopathological features at the tick attachment site (epidermal hyperplasia, hemorrhage, edema, inflammatory cell infiltrate, presence of the feeding cavity and the cementum) agreed with earlier observations (GILL; WALKER¹⁸, 1985; WALKER; FLETCHER²⁵, 1986).

Cutaneous reactions of dogs characterized by a massive neutrophil infiltration, regardless of the number of infestations, confirmed previous observations (THEIS; BUDWISER²⁴, 1974). Moreover, data from the present work also show that following vaccination with crude adult tick extract challenge infestations elicited this same pattern of cellular infiltration and dogs did not seem to be irritated by tick attachment under our experimental conditions. It is known that the dog has the highest neutrophil / mononuclear cell ratio in the blood and the greatest tendency toward involvement of neutrophils in the hematological response to a variety of stimuli (JAIN²⁰, 1986), and moreover GALKOWSKA; OLSZEWSKI¹⁵(1986) have shown that large numbers of neutrophils are present in the lymphatic system in the dog, but not in other species. It has also been shown (COMER¹³, 1988) that acute spontaneous lesions in inflammatory canine skin diseases, such as atopic dermatitis and allergic contact dermatitis, are characterized by pruritus and a cellular infiltration rich in neutrophils, erythema and edema. The lack of such strong reactions in our observations suggests that *Rhipicephalus sanguineus* somehow is able to control the local inflammatory reactions of the dog, because a potentially harmful neutrophilic response does not affect ticks, nor does it cause damage to the hosts skin, nor pain or pruritus. Thus, an important host resistance behaviour pattern - the self grooming - was not seen. The infiltration pattern and the quantitatively less intense reaction are probably linked to the absence of dogs' resistance to this tick species (CHABAUD¹², 1950; SZABÓ et al.**).

On the other hand, hamsters and especially guinea pigs displayed more extense cell infiltration and had a more intense mononuclear cell infiltration. In the case of these hosts, the presence of monocytes in larger numbers, as well as the absence of basophils, could be explained by the late performance of the biopsies. Most probably, reaction to ticks is a very dynamic process with changes in the local cell population throughout the attachment and feeding processes. McLAREN et al.²²(1983) observed a decrease in the numbers of basophils degranulating as early as 18 hours after the attachment of the tick *Rhipicephalus appendiculatus* to sensitized guinea pigs. In addition, DVORAK et al.¹⁴ (1970) explained that basophils are very labile cells, that rupture easily during histological processing.

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Obviously, the parasiting tick species also influences the cellular reaction BROWN; ASKENASE⁹ (1981) observed that pre-sensitized guinea pig reacted to a 24 - hour old tick attachment mainly by mononuclear cells, and to a lesser extent, by basophils when *Rhipicephalus sanguineus* was used. However, when the tick *Amblyomma americanum* was involved, basophils predominated over mononuclear cells.

As for the other cellular types, eosinophils are also commonly present in reactions to ticks (BROWN⁷, 1988). In the data from the present work, the surprisingly high percentage of eosinophils in the skin of hamsters suffering challenge infestations suggests that mechanisms involved in the resistance to ticks might differ, when either vaccinations or previous infestations are used to sensitize hosts.

In summary, hamsters and guinea pigs, hosts which develop significant resistance following repeated infestations or vaccination with crude unfed adult extract react to tick attachment with strong mononuclear or eosinophilic infiltrate, although the presence of basophils can not be ruled out. Dogs, unable to display resistance to this tick, reacted with a PMN neutrophil infiltration, in all experimental conditions. Although the significance of each cellular type in this process is still unknown, work by ALLEN¹ (1973) and BROWN et al.¹¹ (1982) strongly

suggests that basophils are important in the resistance to ticks. According to BROWN⁶ (1985), this cell type could act through the release of histamine causing an increase in the local vascular permeability and a consequently greater delivery of mediators to the tick feeding site. Eosinophils could act by releasing the major basic protein, known to be toxic to helminths, into the feeding cavity, causing damage to ticks. The presence of eosinophils could also be explained by their capacity of inactivating histamine. Lymphocytes and macrophages are probably involved in the immune reaction and in the late inflammatory process. Finally, the persistence of PMN neutrophils in dogs, without demaging the local tissue remains as an intriguing feature in this particular host - parasite relationship.

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RESUMO

Analisou-se, sob microscopia óptica, reação cutânea de cães, hamsters e cobaia à fixação e alimentação do carrapato *Rhipicephalus sanguineus*. Os hospedeiros empregados foram previamente infestados ou vacinados com extrato total de carrapato adulto não alimentado ou eram livres de contato anterior com o carrapato. Biópsias foram obtidas ao final de cada infestação. As alterações comuns a todos os hospedeiros, independente do grupo experimental, incluíram a presença de carrapato ou de suas peças bucais embutidos em um cone de cimento na superfície da pele, hiperplasia da epiderme, hiperqueratose e acantose, edema e infiltração celular inflamatória intensa na derme abaixo do ponto de fixação do carrapato. Os cães, em todos os grupos experimentais, reagiram com um acúmulo praticamente exclusivo de neutrófilos polimorfonucleares enquanto cobaias exibiram uma infiltração celular predominantemente mononuclear nas diversas condições experimentais. Hamsters sofrendo primeira infestação apresentaram uma infiltração predominantemente neutrofílica, infiltração predominantemente mononuclear em resposta a múltiplas infestações e, quando vacinados, uma infiltração preponderantemente eosinofílica. A persistência de neutrófilos nos cães sugere um controle local da resposta inflamatória imune pelo carrapato. A infiltração predominantemente eosinofílica em hamsters vacinados poderia indicar que mecanismos imunes diferentes foram desencadeados pela infestação e vacinação.

UNITERMOS: Histopatologia; *Rhipicephalus sanguineus*; Cães; Hamsters; Cobaias

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