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HISTOLOGICAL RESPONSE OF *EPTESICUS SEROTINUS* (MAMMALIA: CHIROPTERA) TO *ARGAS VESPERTILIONIS* (ACARI: ARGASIDAE)

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ABSTRACT: The sequential histological development of *Argas vespertilionis* (Acarina: Argasidae) feeding sites on *Eptesicus serotinus* (Mammalia: Chiroptera) was evaluated. Neutrophils, followed by Langerhans cells, were the major components of the cellular infiltrate throughout the earliest phase of tick infestation. The host tended to isolate the tick mouthparts by means of a progressive formation of epithelial tissue in the feeding cavity.

Key words: *Argas vespertilionis*, *Eptesicus serotinus*, histological response.

INTRODUCTION

Ticks are obligate blood-feeding parasites of reptiles, birds, and mammals, with a distribution worldwide. Acquired resistance to tick feeding occurs in both mammalian and avian hosts (Johnston and Brancroft, 1918; Dusbábek et al., 1988). Brown and Askenase (1981) demonstrated that the immune response to ticks is dependent on sensitized lymphoid cells or serum components. However, large accumulations of basophils, eosinophils, mast cells, neutrophils, and mononuclear cells have been described as a cutaneous response in hypersensitized hosts (Brown and Knapp, 1981; Gill and Walker, 1985). In addition, interaction between tick salivary gland antigens and specific homocytotropic antibodies bound to the surface of basophils and mast cells produces the degranulation of these cells with histamine liberation (Gill and Walker, 1985). Moreover, the pharmacological mediators released by degranulation of mast cells and basophils are the major effectors of resistance in mammals to ticks (Kemp and Bourne, 1980).

The relationships between ticks and domestic hosts has been thoroughly reviewed by Brown (1988). Host-tick interactions with wild hosts are yet poorly known, but may involve histological responses similar to those described for domestic animals (Bolte et al., 1970; Brain and Bohrmann, 1992). But reptiles, the most ancient host of ticks (Hoogstraal, 1978), have a diffuse inflammatory reaction and granuloma formation (Goldberg and Bursey, 1991).

Argas (*Carios*) *vespertilionis* Latreille is a tick which ranges from West Africa to the Philippines, and is associated with several bat genera resting in caves and rock crevices (Garduer and Molyneux, 1987). Bats seem to be adequate hosts for tick blood ingestion due to the absence of a dense fur cover and the existence of large blood vessels just beneath the dermis (Kunz, 1984). Also, several *Argas* species have long feeding periods for the larval stage (Hoogstraal, 1956); it seems possible that a severe lesion may develop. The long evolution between *A. vespertilionis* and its hosts (Hoogstraal, 1978), together with the features outlined above, make this tick a good choice in the study of the host response at the feeding site of bat Argasid ticks.

Our objective was to describe the cutaneous reaction associated with infestation of *Argas vespertilionis* on a wild population of the bat (*Eptesicus serotinus*). We carried out histochemical studies in order to identify and quantify epidermal Langerhans cells, which may play a role

in the mechanism of resistance against ticks (Allen et al., 1979; Nithiuthai and Allen, 1984).

MATERIALS AND METHODS

Eighteen *Eptesicus serotinus* bats were hand collected in March to July 1991 from a colony living under a corrugated iron roof in Manresa, Spain (41°3'N, 2°15'W). Bats were anesthetised with sodium thiopental (Abbott Laboratories, Madrid, Spain), 20 mg/kg bodyweight, and observed for the presence of attached ticks. Ten bats were selected and killed by cervical dislocation because of the high number of larval *Argas vespertilionis* feeding on them. The skin surrounding tick mouthparts was then finely dissected leaving the undamaged tick in the middle of the skin sample. Samples were classified into three groups on the basis of the time of the attachment, as estimated from the size of larval ticks (Hoogstraal, 1956). Group 1 included 22 ticks with a feeding time of ≤4 days; group 2 contained 24 ticks feeding for 5 to 9 days; group 3 involved 25 ticks just prior to detachment (10 to 12 days). Skin samples taken at sites far away from feeding sites on the forearm were used as controls.

Samples for histology were fixed with 70% ethanol and embedded in either Epon-Araldite mixture (Fluka Chemie AG, Buchs, Switzerland) (Mollenhauer, 1963) or paraffin. To identify the different types of granulocytes, the 1 μm sections were stained with either 1% toluidine blue or panoptic stain (Química Clínica Aplicada S.A., Madrid, Spain). We identified Langerhans cells on epidermal sheets which were immediately adjacent to tick mouthparts. Epidermal sheets were separated from the dermis by incubation in the mixture proposed by Shelley and Juhlin (1977), containing 0.76% ethylenediaminetetraacetic acid (EDTA) (Fluka Chemie AG) (Shelley and Juhlin, 1977). For adenosine triphosphatase (ATP-ase) demonstration the procedure of Robins and Brandon (1981) was followed. Briefly, epidermal sheets were fixed for 60 min at 4 C in 0.3% formaldehyde (Fluka Chemie AG) and then incubated for 60 min at 37 C in the mixture incubation containing adenosine triphosphate (ATP) (Fluka Chemie AG). Finally they were developed in 1% ammonium sulphide for 2 min at 20 C. Control epidermal sheets were incubated in the same medium, but without ATP. Epidermal sheets then were fixed in 2.5% glutaraldehyde (Fluka Chemie AG) and embedded in Epon-Araldite. Ultrathin (400 to 600 Å) sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined under a Jeol 100 B transmission electron microscope (Jeol Ltd., Tokyo, Japan).

To evaluate cellular response, the number of mast cells, basophils, eosinophils, neutrophils and mononuclear cells (fibroblasts, lymphocytes and macrophages) present in 20 fields of 1,000× (0.48 mm² total area) adjacent to tick mouthparts was recorded, as detailed by Gill and Walker (1985). We counted ATP-ase positive cells within the epidermis using a transmission electron microscope as described by Mishima and Matsunaka (1976). Briefly 10 pyramids were cut from each biopsied sample. Seven grids, each having four gold sections, were sampled from each pyramid. The number of ATP-ase positive cells per grid was estimated by counting all fields at 2,000× on each of the grids. For each sample, results were expressed as mean cell by feeding site.

Data were analyzed by the Kruskal-Wallis test (Kruskal and Wallis, 1952) and all means were separated for significance according to a Kolgomorov-Smirnov test (Smirnov, 1939).

RESULTS

Sections stained with the panoptic stain were optimal for differentiation of basophils and eosinophils. Basophil granules were purple and clearly distinct from the bright red eosinophil granules. Epon-Araldite embedded sections stained with toluidine blue were optimal for identification of mast cells, neutrophils and mononuclear cells since these sections afforded greater resolution and revealed more histological detail. Moreover, with this stain, mast cells showed metachromatic granules. Neutrophils were distinguished by their bilobed or multilobed nuclei.

Based on electron microscopy technique of ATP-ase stained sections, we observed an electron-dense precipitate of suprabasally located clear cells in the plasma membrane; these cells lacked tonofilaments, desmosomes, and melanosomes. The contour of these cells was irregular, because of the presence of processes between the keratinocytes (Fig. 1).

For group 1 ticks, the cellular response at tick feeding sites was characterized by cellular infiltration with a marked basophil response (Table 1). The lesion was restrict-

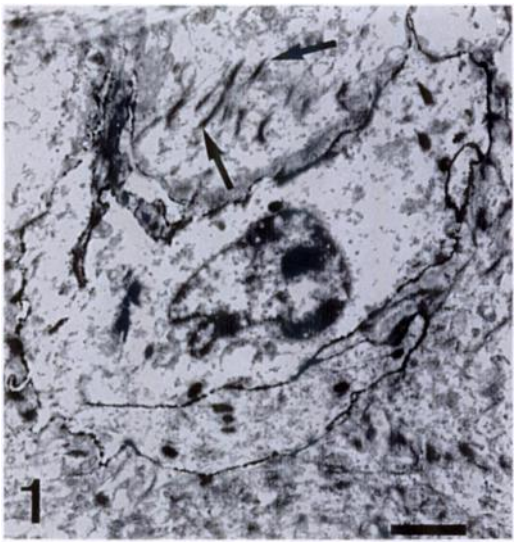

FIGURE 1. Electron micrograph of an ATPase-positive epidermal cell showing an electrondense precipitate in the plasma membrane. The contour of the cell is irregular, because of the presence of processes between the keratinocytes. Tonofilaments (arrows). Bar = 4 μm.

ed to the immediate feeding site with the formation of a dermal feeding cavity. The vessels in the lower dermis had scarcely distributed extravasal and intravasal erythrocytes. Tissue sections were restrict-

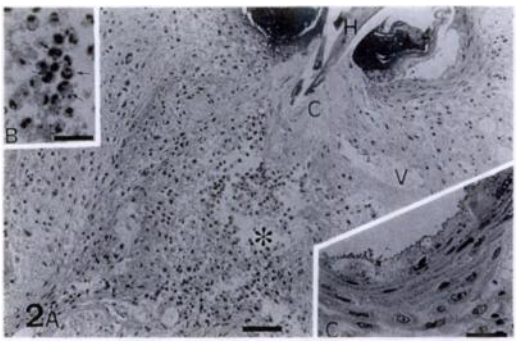

FIGURE 2. Light micrograph of an *A. vespertilionis* feeding site, one to four days after attachment. The cellular infiltrate is patchy (2A). Note in 2B the large number of neutrophils (arrows) infiltrated into the dermis. The epidermis (2C) has evidence of hyperkeratosis. Collagen destruction is also noticed (*). V: blood vessel. C: cement cone. H: hypostome. S: superficial crusting. Toluidine blue. 2A: Bar = 200 μm; 2B: Bar = 30 μm; 2C: Bar = 30 μm.

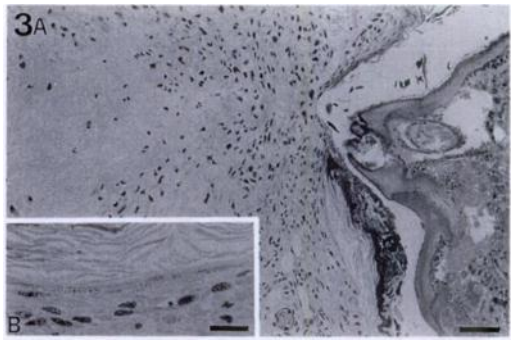

FIGURE 3. Light micrograph of an *A. vespertilionis* feeding site four to nine days after attachment. The dermal lesion is packed and mononuclear cells predominate in cellular infiltrate (3A). The epidermis has evidence of hyperkeratosis (3B). S: superficial crusting. Toluidine blue. 3A: Bar = 200 μm; 3B: Bar = 30 μm.

ed to the feeding site immediately adjacent to the tick mouthparts (Fig. 2). The cellular infiltrate tended to be patchy and contained neutrophils, basophils, eosinophils, mast cells, ATP-ase positive cells and mononuclear cells (Table 1). Neutrophils dominated in the cellular infiltrate (Fig. 2), which was rich in ATP-ase positive cells; however, mononuclear cells occurred only rarely. Basophils and mast cells were located in greatest number in the dermis surface and were seen rarely in the deep dermal infiltrate in which neutrophils predominated. Partially degranulated mast cells and basophils were evident, and granules were frequently noted in the intercellular spaces. Skin had hyperkeratosis with superficial crusting (Fig. 2, Table 2); this was restricted to the area immediately around the feeding site.

Among group 2 ticks, the dermal lesion was packed with cells infiltrated between collagen bundles (Fig. 3). Cellular infiltrates were rich in mononuclear cells and ATP-ase positive cells (Table 1). Basophils infiltrated tick-bite lesion and reached their highest level (Table 1). The skin had intense hyperkeratosis with superficial crusting (Fig. 3, Table 2).

Among group 3 ticks, we observed an intense skin reaction that was confined to

TABLE 1. Cellular response of the three groups of *Eptesicus serotinus* to *Argas vespertilionis* feeding. Cell numbers are expressed as the mean number of cells in 20 fields, for 0.48 mm² total; standard deviation is in parentheses. The ATPase positive cells are expressed as number of cells per mm². Within each row, means not possessing the same superscript are significantly ($P < 0.05$) different by the Kolgomorov–Smirnov test.

Cells	Group 1	2	3	Control
Neutrophils	695 (195)[a]	39 (5.8)[b]	6.4 (2)[c]	1.5 (1)[d]
Mast cells	14.4 (1.1)[a]	15.1 (3.1)[a]	3 (0.9)[b]	0.9 (1)[c]
Basophils	50.8 (13.3)[a]	58.5 (8.7)[b]	47.8 (4.6)[a]	0.1 (0.3)[c]
Eosinophils	43.6 (3.2)[a]	34.7 (6.7)[b]	29.2 (3.9)[c]	0.8 (0.8)[d]
Mononuclear cells	241 (66.8)[a]	528 (86.9)[b]	67 (59)[c]	19.1 (7.4)[d]
ATPase cells	680 (46.2)[a]	819 (47.9)[b]	797 (61)[c]	305.9 (41)[d]

the epidermis and upper dermis. The epidermis developed an intense acanthosis with hyperkeratosis and crusting on the surface (Figs. 4A, 4B; Table 2). Moreover, there was a discrete cellular infiltrate in the superficial dermis composed of mononuclear cells, eosinophils and basophils (Table 1). Usually tick mouthparts were embedded in both a serocellular crust and a reactive epidermal layer which surrounded both the chelicerae and the hypostome (Figs. 4A to 4D). However the deepest hypostome parts were enveloped by rough collagen fibers.

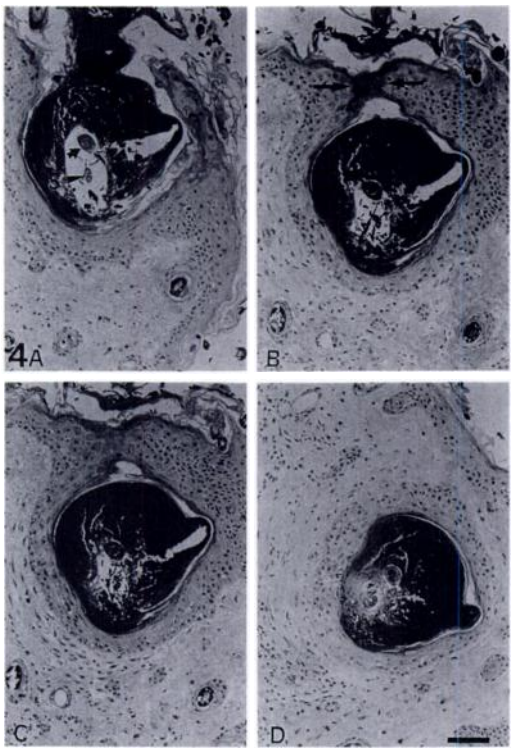

FIGURE 4. Panels A to D include serial sections of the developing epidermal layer surrounding tick chelicera (➡) and hypostome (►). Note the epidermal border fusing to surround the tick mouthparts (arrows in 4B). S: superficial crusting. Toluidine blue. Bar = 200 μm.

DISCUSSION

Ackerman et al. (1981) proposed that immunologically mediated inflammatory reactions render the tick feeding site unfavorable to the tick. However, Gill and Walker (1985) found that host reaction to tick feeding depended greatly on the species of tick and host concerned, the time post-attachment, and whether the host was sensitized. Based on our results with *E.*

TABLE 2. Histological features of cutaneous reactions of *Eptesicus serotinus* at *Argas vespertilionis* feeding sites.

Lesion	Group 1	2	3	Control
Hyperkeratosis	+[a]	++	+++	−
Acanthosis	−	+	+++	−
Superficial crusting	+	++	+++	−
Collagen in feeding cavity	−	+++	+++	−
Epithelium in feeding cavity	−	−	+++	−

[a] Features are coded as −, absent; +, mild; ++, marked; and +++, extensive.

serotinus, a pattern of cellular infiltration occurred during the earliest phase of tick infestation similar to that proposed for other mammals (Gill and Walker, 1985; Latif et al., 1990). Neutrophils were the major component of the cellular infiltrate at an initial stage of tick infestation, and the extent of collagen destruction at tick feeding sites paralleled the degree of neutrophil infiltration. Yet, Junqueira et al. (1980) showed that neutrophils assume their role of collagenase-producing cells earlier than macrophages, fibroblasts, and eosinophils. Thus these polymorphonuclear leukocytes may be involved in the loosening of the collagenous framework that permits both cellular infiltration and a rapid diffusion of factors released by degranulated mast cells and basophils. The presence of the factors released by degranulated mast cells and basophils (such as histamine) appear to be involved in tick rejection (Kemp and Bourne, 1980).

Based on our findings of an increase of ATP-ase cells around tick feeding sites, we propose that during an initial stage of *A. vespertilionis* infestation, *E. serotinus* produces an immune response similar to that described for other tick-resistant mammals (Nithiuthai and Allen, 1984). We assumed that ATP-ase positive cells observed in our results were Langerhans cells, based on their morphology, their position within the epidermis, and their positive staining for ATP-ase, which is considered specific for Langerhans cells under our experimental conditions (Robins and Brandon, 1981). Thus we believe that Langerhans cells may play a role in the acquisition and expression of tick resistance in bats.

Our major histological finding was that the host appeared to separate the tick and the dermal capillaries by means of a progressive increase of the epidermal layer and proliferation of epithelial cells in the feeding cavity. The sequence of cellular events described in the regeneration of dermis after injury includes both collagen degradation with posterior randomly oriented collagen deposition and extensive neovascularization (Murphy et al., 1990). However, in our study the proliferation of epithelial cells in the feeding cavity included degeneration and deposition of collagen bundles, but not extensive neovascularization. Therefore, we propose that *E. serotinus* develops resistance to *A. vespertilionis* infestation by making up a layer of epidermis which interferes with tick feeding.

In most investigated mammals, resistance to ticks is structurally characterized by a significant cutaneous basophil response (Brown, 1988). To our knowledge, this is the first formal demonstration of the proliferation of epithelial cells of the feeding cavity. Thus, we suggest that the proliferation of the epithelial cells in the feeding cavity is an evolutionary acquisition that *E. serotinus* expressed against *A. vespertilionis* feeding; that response may be influenced by the 10 to 12 days (Hoogstraal, 1956) tick feeding time. Since the cutaneous histopathology of bats is poorly known, we cannot exclude the possibility that the observed histopathology is not a specific response to tick feeding, but a generalized reaction to any cutaneous intrusion. This fact encourages us to carry out further experiments in order to elucidate the specific cutaneous response of bats to tick feeding.

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