INFLAMMATORY RESPONSE IN *DERMATOBIA HOMINIS* INFESTED RABBITS

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SUMMARY: Twenty five rabbits divided into 5 groups of five animals were used to investigate the tissue response to larvae of *Dermatobia hominis*. Three groups of animals were previously immunized with extracts of first-, second- and third-instar larvae, respectively, before infestation. The fourth group of animals was not immunized and was infested at the same time as the previous ones and the fifth group was not submitted to any intervention and was used for histological description of normal skin. Skin biopsies were collected from infested animals 1, 3, 5, 7, 9 and 11 days after infestation. The skin samples were embedded in historesin and sections were stained with hematoxylin-eosin, Giemsa and toluidine blue. The effector cells present in the inflammatory exudate were eosinophils, basophils and mast cells. The pattern of inflammatory response was similar in all experimental animals, differing only in time and intensity. In previously immunized animals mononuclear cells appeared in the infiltrate 24 hours after infestation and only on the fifth day after infestation in the only infested animals. Fibroblasts in process of cell division and activated fibroblast surrounded the inflammatory reaction delimiting the inflammatory process around the larva and also the fistulous tract which extended to the epithelium. The results demonstrated that despite the greater intensity the inflammatory process was unable to provoke larval death.

KEY WORDS: *Dermatobia hominis*, eosinophils, mast cells, basophils, rabbits.

INTRODUCTION

Dermatobia hominis is a parasite limited to the American continent. The parasitism of domestic animals is a secondary adaptation, since before the 16th century it only parasitized wild mammals and birds (GUIMARÃES & PAPAVERO, 1966). Today the parasite represents one of the most important causes of myiasis in the Neotropical Region and is particularly outstanding among cuterebrids because of its importance in economic and public health terms (CATTS, 1982).

Although the myiasis caused by *Dermatobia hominis* occur in several species of domestic and wild animals and in man, their economic impact is particularly important in cattle where they cause reduction in weight gain (McMULLIN *et alii*, 1989) and intense devaluation of hides (SANCHO, 1988).

The need to develop alternative techniques for the control of ectoparasites has led to a focusing the research on the understanding of the immunobiology of parasitosis, especially in terms of the development of vaccines (BOWLES *et alii*, 1992). The difficulties of studying these aspects using natural hosts have imposed the use of experimental models that will provide information that may be extrapolated to other species.

In previous experiments, we observed that rabbits immunized with larval extracts of *Dermatobia hominis* developed an immunologic response (MOTA *et alii*, 1980) and that in artificially infected rabbits anti-L1 antibodies can be detected 5 days after infestation (LELLO & BOULARD, 1990). However, since this is a large-sized parasite, the specific antibodies produced may play an adjuvant role in the effector mechanism that mediated damage to the parasite (OLIVEIRA-SEQUEIRA *et alii*, 1996). On this basis, the present study was designed to compare the local cell response of rabbits infested with larvae of *Dermatobia hominis* after immunization with larval antigens and rabbits only infested.

MATERALS AND METHODS

Collection of infective larvae

First-instar *Dermatobia hominis* larvae were obtained in the laboratory by the method of LELLO & PERAÇOLI (1993). Second- and third-stage larvae were obtained from infested cattle by squeezing the parasitic nodules.

Preparation of larval antigens

Larvae of each instar were ground in phosphate buffer, pH 7.2, using a Virtis blender. The homogenate was centrifuged at 4°C for 30 minutes at 15,000 rpm. Protein concentration in the supernatant (total extract) was determined by the method of LOWRY *et alli* (1951). These extracts were used as antigen and were stored at -20°C.

Immunization and infestation schedule

Twenty five Norfolk 2000 rabbits (*Oryctolagus cuniculi*) not previously exposed to the parasite were divided into 5 groups. The rabbits of three groups were immunized with L1, L2 and L3 larval antigens, respectively. Each animal received 3 antigen inoculations at the concentration of 6 mg protein/ml. The first inoculation was by subcutaneous route and consisted of 0.5 ml of antigen emulsified in 0.5 ml complete Freund adjuvant. One week later they were reinoculated with 1 ml antigen without adjuvant by the same route. In the third inoculation, each animal received 0.5 ml antigen through the marginal vein of the ear. Three weeks after immunization these animals were infested with 10 to 12 newly-hatched larvae of *D. hominis*, together with the fourth group of animals, which was not previously immunized. The remaining animals (fifth group) were not submitted to any intervention and were used for a histological description of normal rabbit skin.

Biopsies and histological processing

Skin biopsies at the sites of larval penetration were obtained from each animal of the four experimental groups 1, 3, 7, 9 and 11 days after infestation using a 4 mm punch. Skin biopsies of five normal rabbits were obtained for control. Tissues were fixed in Karnowsky solution (4% paraformaldehyde, 1% glutaraldehyde and phosphate buffer, pH 7.2) and embedded in historesin (glycol metacrylate). A glass knife microtome was used to obtain 2 mm sections which were stained with hematoxylin-eosin, Giemsa and toluidine blue.

RESULTS

Normal skin

The epidermis of control animals not immunized or infested showed two or three cell layers covered with a thin keratin layer. The dermis was relatively thick, with irregular papillae and with dense collagen fibers in the reticular layer. Few mast cells were present among the collagen fibers and around papillary vessels (Fig. 1).

Skin from infested rabbits

After 24 hours and on the third day after infestation, the point of larval penetration was characterized by the lack of continuity of the epithelium, which was filled with an inflammatory exudate rich in cell debris. The adjacent epithelium was thickened and invaded by partially degranulated eosinophils (Fig. 2). Normal or degranulated mast cells were present in the papillary dermis, in addition to numerous basophils (Fig. 3). The blood vessels were congested with many eosinophils and basophils inside them and also in the adjacent connective tissue.

On the fifth day after infestation it was already possible to observe a fistulous tract extending from the point of larval penetration to the site of their installation deep in the dermis. A necrotic zone was visible around the larvae and was surrounded by an inflammatory exudate which presented, in addition to eosinophils and basophils, mononuclear cells and activated fibroblasts as well as fibroblasts in process of cell division. At this moment it was possible to identify the formation of granulation tissue.

Seven days after infestation the orifice of penetration and the fistulous tract were larger. The larvae, deeply located in the dermis, were in the molting phase and released exuviae could be observed. In contrast to the larvae, the exuviae were covered with inflammatory cells (Fig. 4). The inflammatory reaction, containing the same cell constituents, was more exuberant and was clearly delimited by granulation tissue rich in mononuclear cells and fibroblasts which surrounded both the reaction around

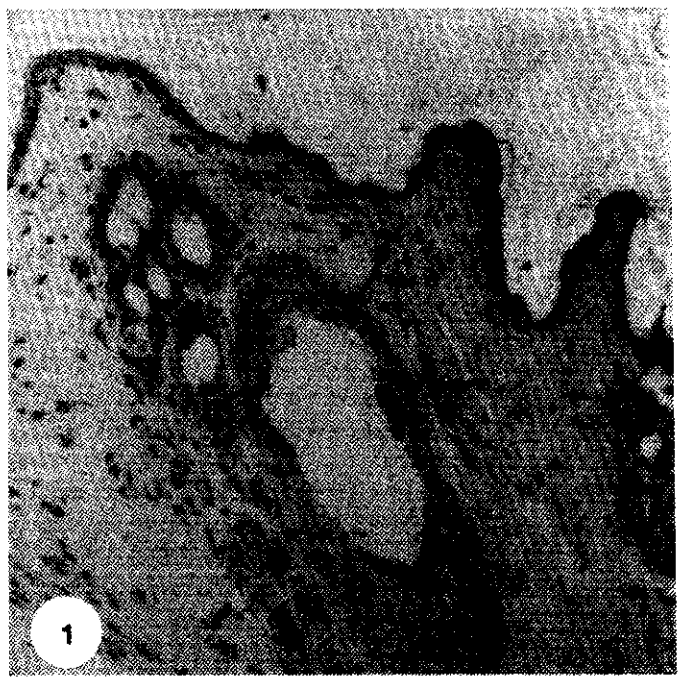

Fig. 1 – Section of rabbit normal skin. Few mast cells in the dermis (arrows). Bar = 50µm.

Fig. 2 – Site of larval penetration 24 hours after infestation. The discontinuity of the epithelium is filled with an inflammatory exudate (*) and the adjacent epithelium is invaded by degranulated eosinophils (arrow). Posterior end of the larva (L). Bar = 40µm.

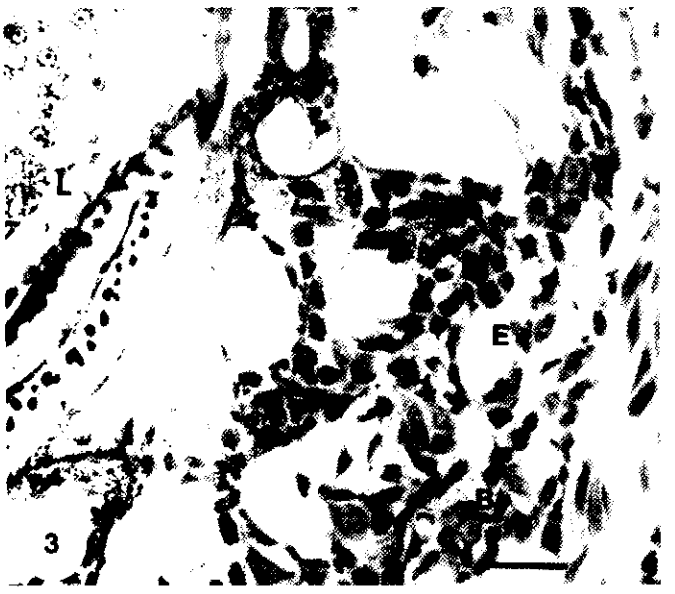

Fig. 3 – Inflammatory reaction around the larva 24 hours after infestation. The empty spaces represent unstained collagen fibers. Larva (L); Eosinophils (E); Basophil (B). Bar = 20µm.

the larva (Fig. 5) and the fistulous tract to the epidermis. No changes in the inflammatory reaction were observed until the 11th day, except for being progressively more extensive.

Skin of infested rabbits after immunization

The sequence of events described for the infested animals was similar to that of immunized/infested animals regardless of the antigen used. The inflammatory reaction was similar but of earlier occurrence and more intense. In contrast to the animals that were only infested, mononuclear cells appeared in the exudate around the vessels 24 hours after infestation. These differences were not sufficient to provoke any kind of damage to the larvae.

Fig. 4 – Inflammatory reaction and granulation tissue around the molted larva (L) 7 days after infestation, showing exuvia studded by inflamatory cells (Arrow). Bar = 50µm.

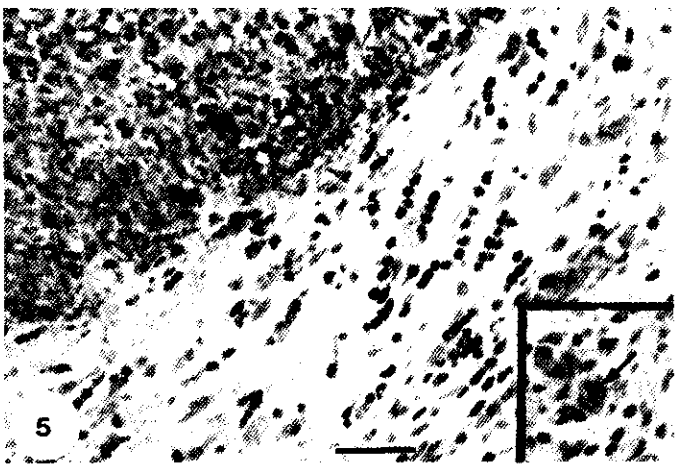

Fig. 5 – Limit of the inflammatory reaction and granulation tissue around the larva 7 days after infestation, showing neoformed vessels (Arrow). Bar = 40µm.

DISCUSSION

Even though anti-*Dermatobia* antibodies are produced by rabbits (LELLO & PERAÇOLI, 1993) and cattle (OLIVEIRA-SEQUEIRA *et alii*, 1996), there is no evidence that they are protective in natural infestations, as also observed for other larvae that produce myiasis (SANDERMAN *et alii*, 1992).

At the site of larval installation in the rabbits, there was a predominance of eosinophils, followed by basophils and mononuclear cells. This pattern is similar to that observed in cattle (OLIVEIRA-SEQUEIRA *et alii*, 1996). It should be pointed out that, within the context of the evolutionary parallelism, both cattle and rabbits may be considered to be recent hosts since, unlike *D. hominis*, they do not originate from the Americas. Nevertheless, considering the response to other ectoparasites and even the response of other hosts to *Dermatobia hominis* (GROGAN *et alii*, 1987), the presence of these cells is practically constant (BOWLES *et alii*, 1992).

The increase in eosinophil number has been associated with increases in basophils/mast cells during the development of acquired resistance to ticks (BROWN *et alii*, 1982), but during the course of infection by *Lucilia cuprina* the increase in eosinophil number is not correlated with the increase in mast cell. (BOWLES *et alii*, 1992). Large numbers of eosinophils, basophils and degranulated mast cells were observed in the skin of cattle infested with *D. hominis* (OLIVEIRA-SEQUEIRA *et alii*, 1996). The tissue basophilia, observed in the present experiment, was more intense than that observed in cattle and, even though we may not consider that this difference alters the role of these cells in the response to *D. hominis*, some investigators consider basophils to be more important than eosinophils in the manifestation of resistance to ticks (BROWN *et alii*, 1982).

Eosinophils play a role on non-phagocytable parasites by releasing the content of their granules (DAVIDSON, 1985). In this type of reaction the presence of antibodies is fundamental since they act as ligands between eosinophils and parasites. In previously immunized rabbits, despite the early occurrence and greater intensity of the inflammatory reaction, the eosinophils were unable to provoke larval death.

OLIVEIRA-SEQUEIRA *et alii* (1996) demonstrated that the immunoglobulins present at the sites of fixation of *D. hominis* larvae on the skin of cattle do not bind to live larvae but bind to dead larvae and exuviae after the molt to second larval instar. These authors suggested that these findings may represent the morphologic manifestation of a scape mechanism that protects the larvae from the response of the host. PRUETT (1993) demonstrated that the digestive enzymes of *Hypoderma lineatum* can break down the IgG of cattle *in vitro*. In any case, the result of both events would be the impairment of antibody binding to the target and consequently the impossibility of a direct action of eosinophils on the larvae.

Although we did not use techniques that would permit the visualization of immunoglobulins in rabbit skin, we observed that eosinophils were only detected adhering to exuviae of larvae in the molting phase, an event probably occurring after opsonization by antibodies.

Among the accessory cells in the response of rabbits to *D. hominis* larvae there was a strong presence of activated fibroblasts and of fibroblasts in the process of cell division. According to GROGAN *et alii* (1987), the fibroblast may play the role of imprisoning the larva, creating a fibrous pustule-like nodule. Furthermore, once activated, these cells function as a stimulus of lymphocytopoiesis and granulocytopoiesis. The architectural arrangement of fibroblasts and their intimate association with eosinophils and mononuclear cells observed in the present study support this hypothesis.

Despite the complex response of the hosts, whose infiltrating cells act in a combined manner in several other parasitoses in which they produce appreciable damage, under natural conditions *D. hominis* larvae survive and undergo metamorphosis, continuing their development. The investigation of the mechanisms involved in the immunologic escape of these larvae is a prerequisite for the future development of immunologic methods of control. Within this context, the use of rabbits as an experimental model offers obvious cost advantages and, in addition, the similarity of the response indicates the possibility of extrapolating the results to other more economically important species.

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SUMÁRIO

Vinte e cinco coelhos, divididos em cinco grupos de cinco animais, foram utilizados para se investigar a resposta tissular às larvas de *Dermatobia hominis*. Animais de três grupos foram imunizados com extratos totais de larvas de primeiro, segundo e terceiro estádio, respectivamente, antes da infestação. Após a imunização estes animais, bem como outros cinco não imunizados, foram infestados com 10 a 12 larvas de primeiro estádio de *D. hominis*. Os cinco outros animais, sem nenhuma intervenção, foram utilizados para obtenção dos parâmetros histológicos da pele normal. As biópsias de pele foram colhidas após 1, 3, 5, 7, 9 e 11 dias após a infestação. As amostras de pele foram incluídas em historresina e secções de 2µm foram coradas pela hematoxilina-eosina, Giemsa e azul e toluidina. O infiltrado inflamatório era constituído, predominantemente, por eosinófilos, basófilos e mastócitos, tanto nos animais imunizados e infestados como nos apenas infestados. A principal diferença observada entre os grupos era o fato de que nos animais previamente imunizados a reação inflamatória era mais intensa e precoce do que nos animais

apenas infestados. Nos primeiros, os mononucleares estavam presentes após 24 horas, enquanto que nos animais infestados, somente a partir do quinto dia da infestação. Além das células efetoras, fibroblastos ativados e em processo de divisão se apresentavam circunscrevendo a reação inflamatória ao redor da larva e delimitando um trajeto fistuloso que se estendia até o epitélio. Apesar da grande intensidade da resposta inflamatória, não foi observada a morte das larvas.

PALAVRAS-CHAVE: *Dermatobia hominis*, eosinófilos, mastócitos, basófilos, coelho.

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