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Inflammatory reaction to the human bot-fly, *Dermatobia hominis*, in infested and reinfested mice

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Abstract. Two groups of mice were infested with first stage larvae of the human bot-fly, *Dermatobia hominis* (Linnaeus Jr) (Diptera: Oestridae). In the first group, skin biopsies were carried out 1, 3, 5, 7, 10 and 18 days after infestation. The second group was also infested but had all the larvae removed 5 days after infestation. The mice in the latter group were reinfested 4 weeks later and skin biopsies were carried out 1, 3, 5, 7, 10 and 18 days after reinfestation. In the first group, an inflammatory reaction began slowly, the neutrophils being the main inflammatory cells, eosinophils being scarce. The reaction progressed with time, developing a necrotic halo around the larvae containing inflammatory cells surrounded by fibroblasts. The inflammation invaded the adjacent tissue. In the second group, the inflammatory reaction was intense on the day immediately after reinfestation, the pattern being changed by the presence of a large number of eosinophils. Activated fibroblasts surrounding the necrotic area around the larvae appeared 3 days after reinfestation in the second group and 7 days after infestation in the first group. The results demonstrated that the previous contact with the antigens elicited the early arrival of eosinophils, probably through the chemotactic factors liberated by mast cells in the anaphylactic reaction.

Key words. *Dermatobia hominis*, eosinophils, inflammation, mice, neutrophils.

Introduction

Dermatobia hominis (Linnaeus Jr), commonly known as human bot-fly, is found in tropical America, from Mexico to Northern Argentina, but not in Chile (Neel *et al*., 1955). Its larva is an obligatory skin parasite of wild and domestic mammals. During the larval period of about 30 days, the larva grows and forms a nodule that is visible on the surface of the skin of the host. Among the domestic animals, cattle are the preferred hosts. This parasitism is a serious problem to the livestock industry; affected animals have their hides damaged, are irritated and do not feed properly, causing loss of weight, a reduction in milk production (McMullin *et al*., 1989) and devaluation of hides (Sancho, 1988).

Alternative techniques for the control of ectoparasites are focusing on understanding the immunology of parasites, especially in terms of development of vaccines (Bowles *et al*., 1992). The difficulties of using natural hosts have imposed the use of experimental models, particularly rabbits. In rabbits, Mota *et al*. (1980) observed that the hosts immunized with larval extracts developed an immunological response. Using the same model, Lello & Boulard (1990) demonstrated that anti-first stage larva antibodies could be detected 5 days after infestation. Lello *et al*. (1999) described the local cellular response of rabbits immunized and/or infested with larvae of *D. hominis*. Using rats, Pereira *et al*. (2001) described the development of skin lesions caused by larvae of *D. hominis*. The present study

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was designed to verify the local cellular response of naïve and reinfested mice to *D. hominis* larvae.

Materials and methods

To obtain *Dermatobia hominis* first stage larvae, heavily naturally infested cattle were kept indoors and mature larvae were collected on the ground each morning. The larvae were placed in plastic boxes containing humid soil to pupate. Adults emerged 30–35 days later and were transferred to a large cage under controlled temperature, humidity and constant light. House flies, *Musca domestica* Linnaeus, were introduced into the cage 2 days after *D. hominis* to serve as egg carriers. The eggs were collected from the carriers' abdomens, deposited on a coverslip and maintained in a humid box at 28°C. The larvae, which hatched 4–5 days later, were collected and used to infect the experimental mice (Lello & Peraçoli, 1993).

Sixty out-bred male Swiss mice (*Mus domesticus domesticus*), two months old and weighing about 30 g, were used as hosts. One group of 30 mice was infested with four to five larvae each. Mice from this group were sacrificed, five at a time on days 1, 3, 5, 7, 10 and 18 after infestation. Biopsies were taken from the sites of infestation. A second group of 30 mice was similarly infested and all larvae were removed 5 days after infestation, by punch biopsies carried out under anaesthesia. After 4 weeks these mice were reinfested and then sacrificed, five each time, again on days 1, 3, 5, 7, 10 and 18 after reinfestation. Biopsies were taken from the sites of reinfestation. Skin biopsies were fixed in McDowell (50 ml of 1% glutaraldehyde in 0.1 M pH 7.2 phosphate buffer, 20 ml of 4% paraformaldehyde, 30 ml 0.2 M pH 7.2 phosphate buffer), progressively dehydrated and embedded in hydroxyethyl methacrylate plastic (Polyscience, Warrington, PA). Sections of 2 μm thickness were stained with Haematoxylin–Eosin (HE), 6% Giemsa in 0.1 M, pH 7.0 phosphate buffer, and 0.25 Toluidine Blue in McIlvaine buffer pH 2.0 and pH 7.0.

Results

Primary infestation

One day after infestation, at the point of larval penetration, a discontinuity of the epithelium with hyperplasia at the borders was observed. From the site of larval penetration to its lodging place, a fistulous tract was observed, limited by inflammatory cells. The larva was found in the area of the subcutaneous muscle, surrounded by a discrete inflammatory reaction with neutrophils and few eosinophils. The vessels around the larva were dilated, containing neutrophils. A few intact and many degranulated mast cells were observed scattered in the dermis (Fig. 1A).

On the third day after infestation, an increase in intensity of inflammatory reaction was observed. The fatty tissue, as well as the loose tissue below the subcutaneous muscle, was invaded by inflammatory cells, predominantly neutrophils. Around the larva, necrotic tissue was observed, composed mainly of disintegrated neutrophils (Fig. 1B).

The same pattern was observed on the fifth day; however, it was more intense, with increased numbers of eosinophils, principally around the vessels near the parasite. A few elongated fibroblasts were observed surrounding the necrotic tissue next to the larva.

On the seventh day, the larva, in the subcutaneous tissue, had already started to moult to second stage and, while no inflammatory cells were at any moment adhering to the larval cuticle, in this period they appeared to be in intimate contact with the exuvia (Fig. 1C). The orifice, at the larval penetration point, and the fistulous tract were enlarged. On the edges of the orifice, the epithelium was hypertrophied. The inflammatory reaction was extended to the adjacent tissues, in which neutrophils and mononuclear cells were present along with fibroblasts that were elongating to limit the inflammatory reaction around the larva. In the inflamed tissue adjacent, there were mononuclear cells near newly formed vessels. A great quantity of degranulated mast cells appeared on the superficial dermis.

Ten days after infestation, the larva had grown a great deal, and the tumour produced by it could be seen macroscopically. The area of necrosis around the larva had increased. A ring of fibroblasts could be seen isolating the necrotic halo of the inflammation, which was extended in all directions in the adjacent tissues, reaching even to the dorsal muscle. In the inflammatory reaction, mononuclear cells, especially plasma cells, were predominant. In the loose tissue, far from the larva, an accumulation of mononuclear cells was observed around the vessels and nerve fibres (Fig. 1D).

On the eighteenth day after infestation, the larva had moulted to the third stage. The original exuvia could be observed, studded with inflammatory cells. Several layers of fibroblasts separated the necrosis from the area of inflammation, which was extended in all directions, presenting degenerating polymorphonuclear cells and many mononuclear cells.

Reinfestation

One day after reinfestation, the number of mast cells in the subepithelial tissue appeared to be only slightly larger than in the case of the animals that had received a single infestation. The inflammatory reaction around the larva, however, was far more intense than in the first group for the same period, also showing an invasion of inflammatory cells in the fatty tissue, subepithelial and loose subcutaneous tissue. Following the single infestation neutrophils were the predominant cells, but with reinfestation eosinophils were markedly predominant. Mast cells were observed scattered among these cells (Fig. 2A).

On the third day after reinfestation, this reaction was even more intense, with greater invasion of adipose, subepithelial and subcutaneous tissues, making the intercellular spacing even smaller. Besides neutrophils and great

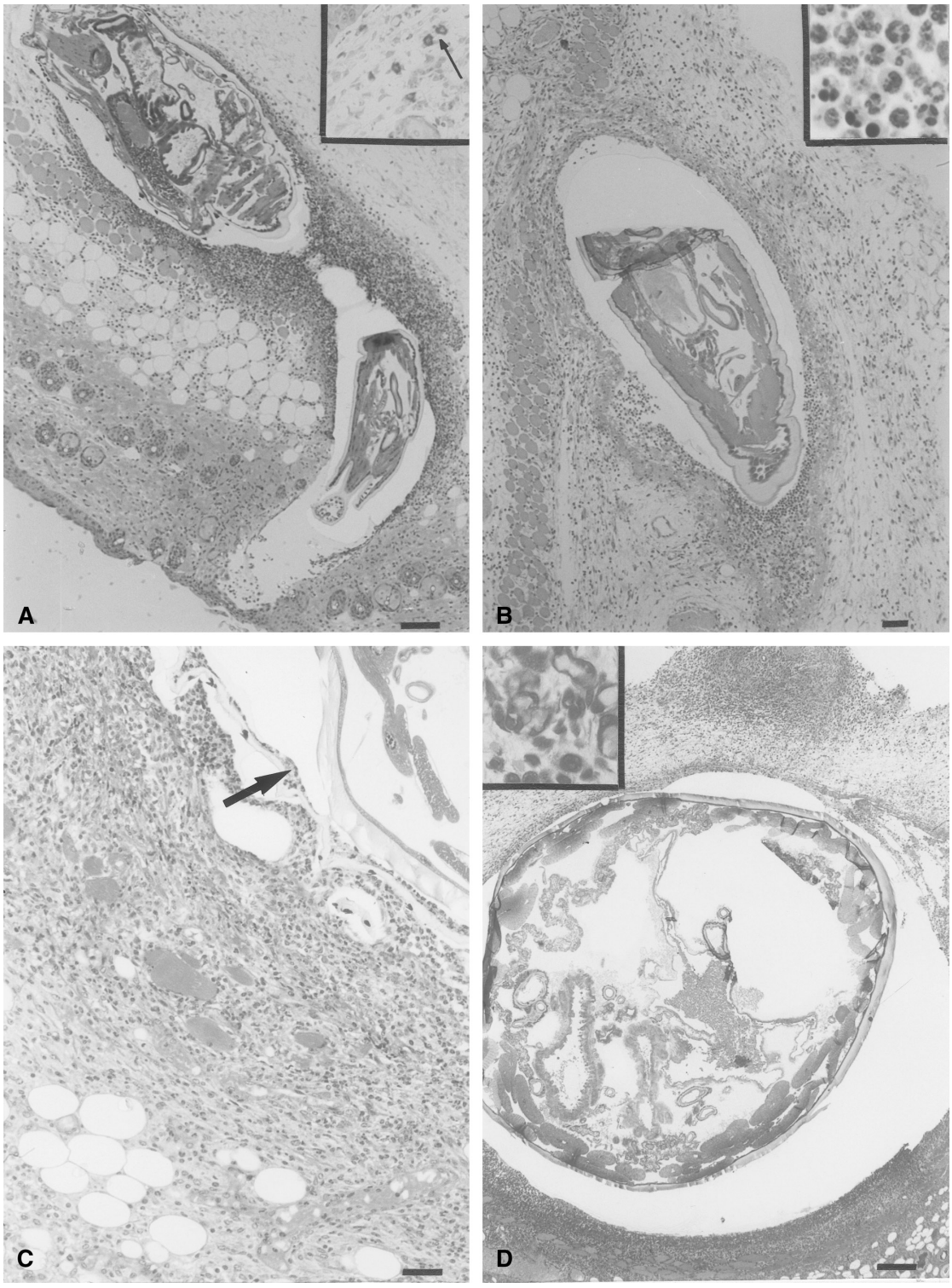

Fig. 1. (A) Section of mouse skin 24 h after infestation. Fistulous tract and neutrophils infiltrate around the larva. Inset: mast cells in the dermis (arrow). Bar: 50 µm. (B) Section of mouse skin 3 days after infestation. Predominance of neutrophils in the inflammatory reaction (inset). Bar: 50 µm. (C) Section of mouse skin 7 days after infestation. Partial view of the inflammatory reaction around the larva. Exuvia studded with inflammatory cells (arrow). Bar: 50 µm. (D) Section of mouse skin 10 days after infestation. Partial view of inflammatory reaction around the larva. Bar: 100 µm (4 mm). Inset: newly formed vessels and mononuclear cells in the inflammatory reaction far from the larva. Bar: 30 µm.

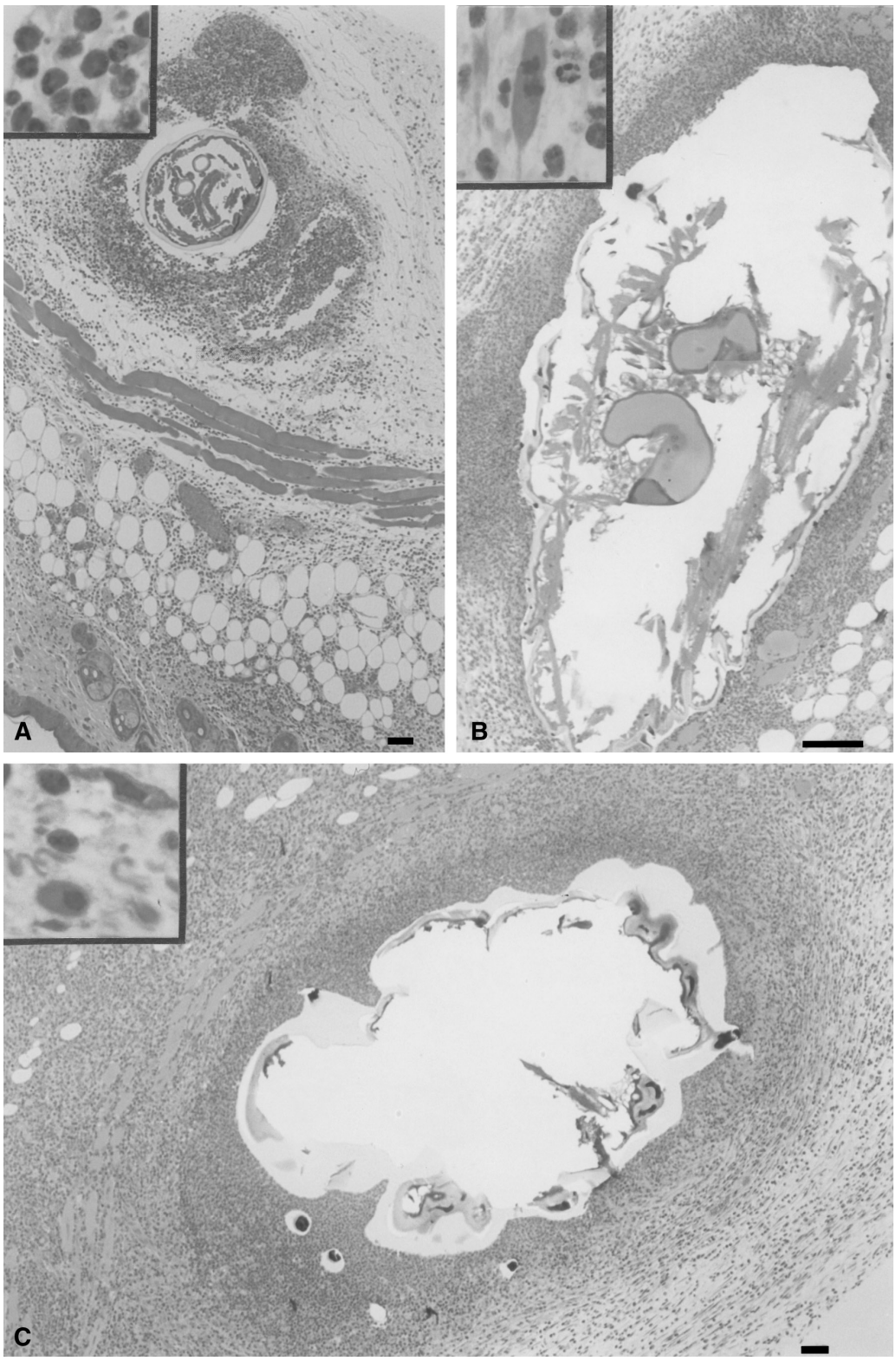

Fig. 2. (A) Section of mouse skin 24 h after reinfestation. Intense inflammatory reaction around the larva predominantly eosinophilic (inset). Bar: 50 μm. (B) Section of mouse skin 3 days after reinfestation. Fibroblast activity around the inflammatory reaction (inset). Bar: 100 μm. (C) Section of mouse skin 5 days after reinfestation. Inflammatory reaction surrounded by many layers of fibroblasts. Inset: plasma cells far from the larva. Bar: 100 μm.

quantities of eosinophils, there was an increase of the necrotic halo around the larva, in the number of mononuclear cells in the adjacent tissue below and in fibroblast activity, preparing to isolate the larva and the inflammatory reaction involving it (Fig. 2B).

The same pattern was observed on the fifth day, with even greater intensity. Some layers of fibroblasts surrounded the necrosis. The quantity of mononuclear cells in the inflammation had increased and it was possible to see plasma cells at some distance from the larva and newly formed vessels beyond the necrotic area. In some of the larvae, the detached cuticle could be observed, indicating the start of moulting. In this region, the inflammatory cells were attached to the future exuvia (Fig. 2C). On the seventh day after reinfestation, the necrosis was covered by several layers of fibroblasts. The number of newly formed vessels and of mononuclear cells in the region was greater when compared to the same period of the prime infestation group. The pattern of inflammation observed on the tenth and eighteenth day after reinfestation was similar to the ones observed before, but more intense. Some of the biopsies showed that the lymphatic nodule neighbouring the infestation region was enlarged and infiltrated by many mast cells.

Discussion

The characteristic lesion provoked by *D. hominis* larvae observed in the skin of infested mice was similar to those described in other hosts (Lello *et al*., 1980; Oliveira-Sequeira *et al*., 1996; Lello *et al*., 1999; Pereira *et al*., 2001). A fistulous tract appeared, from the larval penetration point to the final lodging place, bounded by inflammatory cells. Oliveira-Sequeira *et al*. (1996), studying *D. hominis* infestations in cattle, described the presence of antigens of first and second stage larvae in the fistulous tract. The same authors suggested that the tract must be maintained by substances secreted and/or excreted by the larvae that act as irritants to prevent healing and consequently maintain communication of the larvae with the exterior, permitting gas exchange.

Epithelial alterations such as intercellular oedema, vacuolization and degeneration of epithelial cells, and formation of vesicles and micro-abscesses have been reported previously to be provoked by dipterous larvae (Oliveira-Sequeira, 1992) and by ticks (Allen *et al*., 1977; Brown, 1988; Fivaz *et al*., 1991). These alterations appear to be common in dermal pathology and are not characteristic of any one particular parasite (Muller *et al*., 1983).

Differing from the response observed in cattle (Oliveira-Sequeira *et al*., 1996) and rabbit (Lello *et al*., 1999), where the main cell types found were basophils and eosinophils, in the mice studied here the presence of basophils was not observed. In the animals infested only once, the presence of a great number of mast cells was observed, mainly in the subepithelium; neutrophils were the main cells of the inflammatory reaction, as observed by Pereira *et al*. (2001) in infested rats. Although no mast cell count was made, these cells were present throughout the study, at all examinations and appeared to increase in number as infestation progressed, being even more abundant and degranulated in the reinfested animals. These data agree with those of Ushio *et al*. (1993) in tick-infested mice. Oliveira-Sequeira (1992) observed, in experimental infestation of cattle with *D. hominis* larvae, that immediately after infestation and in the following 24 h, irritation of the host was visible, demonstrated by attempts to remove the larvae by licking the areas where larvae had been deposited. This was attributed to cutaneous hypersensitivity characterized by cutaneous mast cell-dependent eosinophilia.

Absence of basophils in the skin of the *D. hominis* infested mice was balanced by the great number of mast cells. The increase in eosinophil number was associated with increase in basophils/mast cells by Brown *et al*. (1982) during the acquired resistance to ticks, but not by Bowles *et al*. (1992) during the course of infection by *Lucilia cuprina*. Our results suggest that the neutrophil reaction to the initial infestation is a non-specific response of innate immunity. The reaction to the second infestation, rich in eosinophils is associated with acquired immunity. 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 because they act as ligands between the cells and the parasites. Lello & Peraçoli (1993) demonstrated the production of anti-*D. hominis* antibodies by rabbits. In previously immunized rabbits, Lello *et al*. (1999) described an early occurrence and greater intensity of eosinophils in the inflammatory reaction but which was unable to provoke larval death. Oliveira-Sequeira *et al*. (1996) demonstrated that the immunoglobulins detected in the location of *D. hominis* larvae on the skin of cattle do not bind to the live larvae but bind to the dead larvae and exuviae. These data lead to the conclusion that despite the intense immune response of the host, the parasite probably possesses an efficient mechanism of escape, by secreting substances capable of annulling the effects of immune response.

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