

Functional Characterization of Neutrophils from Nine-Banded Armadillos (*Dasypus novemcinctus*; Edentata, Dasypodidae) Infected with Microfilariae

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Abstract: The neutrophils from uninfected armadillos have adequate phagocytic and bacteriocidal activity, but diminished chemotactic activity. However, endocytic activity from armadillo neutrophils infected with bacteria, parasites or viruses is unknown. This report shows that neutrophils from armadillos infected with microfilariae displayed deficiencies in their bacteriocidal mechanisms and TNF-like production, but not in their endocytic capacity.

Key words: Armadillo, *Dasypus novemcinctus*, microfilariae, neutrophils

INTRODUCTION

Wild animals are susceptible to a great number of bacteria, parasites and viruses, which can be acquired through bites by infected arthropods or by the consumption of contaminated water or cadavers. The filarial parasites are nematodes which infect several vertebrates and which live in epithelium tissues or lymphatic tissues. The mechanisms by which circulating microfilariae avoid host immune response are poorly understood^[1]. However, the protease inhibitors from parasitic agents inhibit a diverse range of host functions such as chemotaxis of neutrophils, blood coagulation and antigen processing^[2-4]. In filarial nematodes, the presence of serine proteinase inhibitors has been reported which inhibit enzymatic activity of cathepsin G and elastase from human neutrophils^[5]; these enzymes have several functions such as: antimicrobial activity, degradation of extracellular matrix, chemoattractant for monocytes and T lymphocytes and mitogenic activity in T lymphocytes^[6-8].

During research on armadillo immune response, we observed infected armadillos with microfilariae in their bloodstream (Fig. 1). This report observes some deficiencies in neutrophil activity in two nine-banded armadillos infected with microfilariae such as: levels of tumor necrosis factor-like activity (TNF-like) and phagocytic activity (endocytosis, NBT reduction and intracellular death).

MATERIAL AND METHODS

Nine-banded armadillos were captured in the state of Guerrero in Mexico. Twenty milliliters of peripheral blood were collected by means of cardiac puncture from the animals who had previously been anesthetized with ketamina base. The purification of the leukocytes

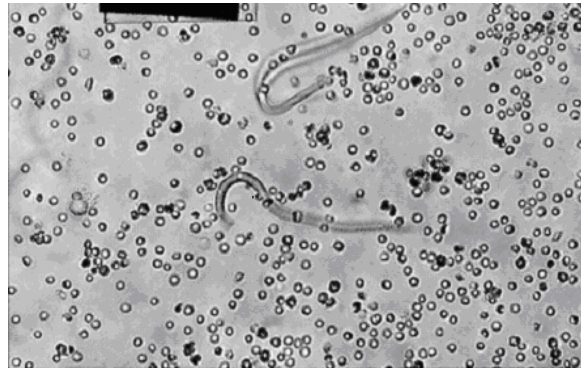


Fig. 1: Microfilariae in the bloodstream of nine-banded armadillos

was carried out by the dextran technique as we described previously^[9]. The leukocytes were placed in tubes which contained 2.5 ml of Hystopaque ($\delta=1.077$; Sigma Chemical CO., St Louis Missouri, USA), centrifuged at 300 x g 30 min and recovered and washed once with PBS (300 x g for 8 min). The neutrophils were then resuspended in RPMI-1640 medium (Sigma) [with 10% fetal calf serum (Sigma)], without antibiotics. Finally, cellular viability was evaluated using the trypan blue exclusion technique. The purity and viability of the mononuclear cells as neutrophils for each assay were >92%.

The degree of endocytosis of the neutrophils was carried out using total blood and was based on ingestion of FITC-labelled *Escherichia coli* and their detection through flow cytometry as we have already described^[9]. The percentages of NBT reduction of the neutrophils were evaluated in glass coverslips. The neutrophils adhered to coverslips were put in contact with opsonized yeast (*Sacharomyces cereviceae*) and NBT solution (0.15M); after 90 min of incubation time, the percentage of cells with reduction of NBT was

Table 1: Several deficiencies of neutrophils from armadillos infected with microfilariae

Assay of	Armadillos infected with microfilariae (n=4)	Armadillos not infected with microfilariae (n=8)
Endocytosis ^b	96.05 ± 0.34% ^a	91.42 ± 4.08% ^a
NBT reduction ^c	9.0 ± 5%	70.5 ± 18.8%
Intracellular death ^d	50.0 ± 5 %	73.06 ± 16%
TNF-like activity ^e	42.23 ± 8.69%	51.68 ± 1.82%

^aMean and standard deviation

^bPercentages of neutrophils that ingested FITC- labelled *E.coli* evaluated by cytometer flow.

^cPercentages of neutrophils that reduced the nitroblue tetrazolium salt (NBT) after ingesting yeasts

^dPercentages of *Staphylococcus aureus* death after incubation with armadillo neutrophils.

^ePercentages of cytotoxicity of culture supernatants of armadillo neutrophils stimulated with PMA

evaluated by observation under microscopy as Rojas-Espinosa and et al have described^[10]. The bactericidal activity of the neutrophils was carried out by means of the survival of *Staphylococcus aureus* after 30 min of incubation time with the armadillo neutrophils^[11]. The bacteria-neutrophils mixture was centrifuged at 300 x g by 8 min. The pellet fraction was washed twice and resuspended in 1 ml of heparinized saline solution (hss). The pellet was then washed again with 1 ml of hss with added lysostaphin (1 µg/ml). Then, 1 µl of phagocytes was deposited to 999 µl of distilled water. Ten microliters of phagocytes were deposited in plates of soybean-tripticase agar. The plates were incubated overnight and viable bacteria were determined by colony counts. Armadillo neutrophils (1x10⁶ cells/well) were stimulated with PMA for the detection of TNF-like activity in culture supernatants carried out by cytotoxicity bioassay using L-929 cells as we described previously^[12].

RESULTS

No differences were found regarding the percentages of endocytosis between neutrophils from armadillos infected with microfilariae and neutrophils from uninfected armadillos as shown in the table. However, a significant difference was observed in the percentages of NBT reduction in neutrophils from armadillos infected with microfilariae. Intracellular death of *S. aureus* by neutrophils from armadillos infected with microfilariae was also considerably low compared to neutrophils from uninfected armadillos. In the same way, TNF-like activity from neutrophil culture supernatants from armadillos infected with microfilariae was low when compared with TNF-like activity obtained in culture supernatants of neutrophils from uninfected armadillos.

DISCUSSION

These results showed that neutrophils from the armadillos infected with microfilariae show deficiencies in their bacteriocidal mechanisms, but not in their endocytic capacity. In this study, the loss of enzymatic activity by armadillo neutrophils was reflected by its inability to destroy *S. aureus*. We also observed that oxygen-dependent mechanisms were affected, possibly by a low generation of highly

reactive oxygen species or by the inhibition of the mieloperoxidase system (evaluated by NBT reduction). TNF-like production was also affected in neutrophils from armadillos infected with microfilariae. A lower production of TNF could lead to poor activation of T lymphocytes and therefore avoid host immune response. Finally, a deficiency in the bacteriocidal mechanisms of neutrophils, monocytes or macrophages from armadillos infected with microfilariae will allow the establishment of other germs. Given the susceptibility of armadillos to experimental infection with *Mycobacterium leprae*, it would be interesting to know if the phagocytic cells show alterations in the bacteriocidal mechanisms to allow infection by this bacterium to become established, contrary to what happens with other animal models such as the mouse.

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