



Partial characterization of phagocytic activity in neutrophils of the nine-banded armadillo *Dasypus novemcinctus*

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Abstract

Though the armadillo is important as a research model in leprosy studies, the activity of armadillo's neutrophils is an aspect of little research. The aim of this study was carried out to partially characterize the chemotaxis, endocytosis and bacteriocidal ability of the neutrophils found in the nine-banded armadillo (*Dasypus novemcinctus*). Results showed that the chemotactic activity of the neutrophils, evaluated by the movement of the neutrophils through a nitrocellulose membrane (5 µm) in response to a chemo-attractive substance, was greater towards the armadillo serum (5.16 ± 1.35 migration index, $p < 0.05$) than towards the formil methionyl leucil phenylalanine (fMLP, 1.43 ± 0.18 migration index) or human serum (0.56 ± 0.18 migration index). Regarding endocytic capacity of the neutrophils and the monocytes against *Escherichia coli* was evaluated by a flow cytometry and using opsonized and non-opsonized *E. coli*-FITC at the following incubation times: 5, 10, 20, 30 and 60 min. The largest percentage of endocytosis by the neutrophils was $92.32 \pm 0.12\%$ with opsonized bacteria and $77.73 \pm 14.33\%$ with non-opsonized bacteria at 10 min incubation time, while the largest percentage of endocytosis by monocytes was $89.94 \pm 1.40\%$ with opsonized bacteria and $73.07 \pm 15.6\%$ with non-opsonized bacteria at 20 min incubation time. Evaluation of the bacteriocidal capacity of neutrophils using the methyl-thiazol-tetrazolium salts (MTT) reduction color-measurement assay showed an $89.0 \pm 10\%$ mortality rate of non-opsonized *E. coli* and $89.0 \pm 10\%$ of opsonized *E. coli*. In conclusion, the armadillo neutrophils show a good phagocytosis and bacteriocidal activity; however, a deficiency in the migration towards the fMLP was observed. This deficiency could be a cause so that the armadillo neutrophils do not respond quickly to invading microorganism. © 2005 Elsevier B.V. All rights reserved.

Keywords: Phagocytic activity; *Dasypus novemcinctus*; Animal models; Armadillo; Leprae

1. Introduction

Neutrophils and cells of the monocyte-macrophage series are cells with phagocytic activity. Neutrophils form 60–75% of the blood's leukocytes in the majority

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of carnivores, but in other mammals such as the horse they only represent 50% of the leukocytes, while in ruminants like cows, sheep and in rodents, the percentage of neutrophils varies from 20 to 30% (Oliver, 1978; Tizard, 1998). In armadillos, which are used as an experimental model for the study of leprosy, the percentage of neutrophils is of 33% in captivity and 57% in their natural environment (Amescua et al., 1984).

Neutrophils are the first cells to accumulate in tissues during acute inflammation with objective to kill invading microorganisms (Rojas et al., 1996). Although chemotaxis, endocytosis and nitro-blue tetrazolium reduction levels in neutrophils of leprosy patients have been reported (Drutz et al., 1974; Sher et al., 1978), bacteriocidal mechanisms of these cells are poorly known. Only a defect in chemotactic activity was found in leprosy patients (Sher et al., 1978). Armadillo model could be useful to discriminate against if there exists a deficiency in neutrophil activity, as well as to explain the mechanisms for which it passes from acute inflammatory process to a chronic.

In spite of the above-mentioned, the armadillo's immunobiology is a little studied aspect. Beside that, chemotaxis, phagocytosis and bacteriocidal activities in the neutrophils in the different animal species vary depending on the arsenal of receptors and toxic substances that show these cells (Brousseau et al., 1999). Due to this, the aim of this research was to evaluate the chemotaxis, phagocytosis and bacteriocidal capacity of the neutrophils of the nine banded armadillo *Dasypus novemcinctus*.

2. Method and material

2.1. Animals

Nine banded armadillos were captured in the state of Guerrero, Mexico, and were transported to Mexico city where they were put in quarantine.

2.2. Experimental design

The armadillos were anesthetized with ketamine base (ketalar 35 mg/kg weight, 1.5 mL) via intramuscular injection and 20 mL of blood was obtained via

cardiac puncture. The blood was distributed as follows: 3 mL of blood were put in a test tube containing 10 μ L of heparin, which was then used to perform the phagocytosis assay, the remaining 17 mL was distributed in tubes that contained 6 mL of Alsever (6 mL of blood per tube) which were used to perform the chemotaxis and bacteriocidal capacity assays.

2.3. Reagents

Heparin was used at a concentration of 5000 IU/mL (Inhepar PISA, México). *Escherichia coli* bacteria: The *E. coli* ATCC 25922 strain was cultured for 24 h at 37 °C in BHI broth. Afterwards, it was divided in two parts. One part was inactivated with formaldehyde at 10% in saline solution for 24 h and the other part was not inactivated. The inactivated *E. coli* bacteria were washed three times with saline solution (0.85%). Following this, 5 mL of FITC (2 mg/mL) in carbonate–bicarbonate buffer was added and the samples were incubated for 24 h at 4 °C. Later, they were dialyzed with saline solution. The FITC-labelled *E. coli* bacteria were once again separated in two parts, one of which was opsonized by adding 1 mL of decompemented armadillo serum and incubating the sample for 30 min at 37 °C in the dark. After, two washings were conducted with PBS. Then the FITC-labelled opsonized bacteria as well as the FITC-labelled non-opsonized bacteria were adjusted to tube number 2 of the McFarland nephelometer. They were aliquoted and frozen at –70 °C until the time they were used. These bacteria were used for phagocytosis assay evaluated by cytometry flow.

The non-inactivated *E. coli* bacteria were divided in two parts. One part was opsonized as it was described in the previous paragraph and the other was not opsonized. As much the opsonized-*E. coli* as the not opsonized-*E. coli*, the CFU/mL number was determined in soy-tryptocasein agar plates. Then the bacterial suspension was aliquoted and frozen at –70 °C until the time they were used. These bacteria were used to evaluate bacteriocidal activity of armadillo neutrophils.

Chemotactic factors: *N*-Formil methionyl leucil phenylalanine (fMPLP) in DMSO at stock concentration of 10^{-2} M.

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