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Blood and inflammatory cells of the lungfish *Lepidosiren paradoxa*

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Abstract

A special interest exists concerning lungfish because they may possess characteristics of the common ancestor of land vertebrates. However, little is known about their blood and inflammatory cells; thus the fine structure, cytochemistry and differential cell counts of coelomic exudate and blood leucocytes were studied in *Lepidosiren paradoxa*. Blood smear analyses revealed erythrocytes, lymphocytes, monocytes, polymorphonuclear agranulocytes, thrombocytes and three different granulocytes. Blood monocytes and lymphocytes had typical vertebrate morphology. Thrombocytes had large vacuoles filled with a myelin rich structure. The polymorphonuclear agranulocyte had a nucleus morphologically similar to the human neutrophil with no apparent granules. Types I and II granulocytes had eosinophilic granules. Type I granulocytes had round or elongated granules heterogeneous in size, while type II had granules with an electron dense core. Type III granulocyte had many basophilic granules. The order of frequency was: type I granulocyte, followed by lymphocyte, type II granulocyte, monocyte, polymorphonuclear agranulocyte and type III granulocyte. Peroxidase localized mainly at the periphery of the granules from type II granulocytes, while no peroxidase expression was detected in type I granulocytes. Alkaline phosphatase was localized in the granules of type II granulocyte and acid phosphatase cytochemistry also labelled a few vacuoles of polymorphonuclear agranulocyte. About 85% of the coelomic inflammatory exudate cell population was type II granulocyte, 10% polymorphonuclear agranulocyte and 5% macrophages as judged by the nucleus and granule morphology. These results indicate that this lungfish utilises type II granulocytes as its main inflammatory granulocytes and that the polymorphonuclear agranulocyte may also be involved in the inflammatory response. The other two granulocytes appear similar to the mammalian eosinophil and basophil. In

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summary, this lungfish appears to possess the typical inflammatory granulocytes of teleosts, however, further functional studies are necessary to better understand the polymorphonuclear agranulocyte.

Keywords: Lungfish; *Lepidosiren paradoxa*; Blood leucocytes; Granulocytes; Inflammatory cells

1. Introduction

Lungfish are from the Osteichthyes class and Sarcopterygii subclass, a survivor of a once dominant group [1]. These fishes are considered ''living fossils'' and may still possess characteristics of the common ancestor of land vertebrates [2]. Thus, particular interest exists concerning the lungfish and its relatives with regard to vertebrate evolution [1–3]. Little is known about the blood cells of the Sarcopterygii. Of the few articles published, only one has characterised, at the ultrastructural level, blood cells of the Australian lungfish (*Neoceratodus forsteri*) [4]. The fine structure [4] and histochemistry [5] of monocytes, thrombocytes, lymphocytes, neutrophils, eosinophils, heterophils and basophils have been described and there is a previous attempt to explore the inflammatory response of *N. forsteri* which found no difference in the coelomic cell population after lipopolysaccharide injection [5]. Finally, there is an article characterising granulocytes of the South American lungfish (*Lepidosiren paradoxa*), which is mainly related to haematopoietic tissue [6], however, in this report (contrary to the work of Hine et al. [4,5]), only three types of granulocytes are described [6]. Here the morphology, fine structure, and peroxidase and phosphatase localisation of blood leucocytes from *L. paradoxa* and the inflammatory granulocyte population after thioglycollate coelomic stimulation are described. A better understanding of these cells may shed light on fish granulocyte heterogeneity [7] and on the evolution of tetrapod leucocytes.

2. Material and methods

2.1. Lungfish, blood harvesting and leucocyte separation

Lepidosiren paradoxa was captured by local fishermen in the flooded regions surrounding the city of Belém, PA, Brazil. Ten animals, varying from 30 to 70 cm in length, were maintained in water tanks (150 × 75 × 60 cm) on a local farm. Lungfish were maintained in good health by feeding with small chunks of raw fish once a week with no animals dying over a two-year period. A week before blood harvesting, animals were transferred to separate tanks (30 × 20 × 15 cm) and kept at the animal facility in the Instituto Evandro Chagas, Belém. Lungfish were restrained manually; 1 ml of blood was collected by cardiac puncture into 1 ml syringes and added to a tube containing EDTA as anticoagulant (0.5% final concentration). Blood smears from all lungfishes were fixed with absolute methanol and stained with Giemsa for leucocyte morphological classification and counting. Animals were re-bled after 15 days only for ultrastructural studies where blood leucocytes from each individual fish were separated from erythrocytes as described [8].

2.2. Fish coelomic exudate leucocytes

In order to determine the inflammatory blood granulocyte, 1 ml of thioglycollate (3% aqueous solution of Brewer's thioglycollate medium (Sigma), was injected intracoelomically into three fish. These were killed by a blow to the head after 6, 36 and 48 h and a coelomic wash was performed with Dulbecco's Modified Eagle's Medium (DMEM). Briefly, in 60 cm body size animals, without pulling back the skin, 10 ml of DMEM was injected into the coelomic cavity of the fish. The cell suspension was collected in the same syringe, dispersed in tubes disposed on ice and smears performed. The cell suspension was centrifuged at 500 g for 10 min at 4 °C and fixed for transmission electron microscopy.

2.3. Light microscopy

Giemsa stained blood smears were examined and photographed under a Zeiss Axiophote microscope using the immersion 100× objective. After leucocyte classification, differential counts were made for each blood smear of 10 fish