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The Ultrastructure of the Camel Eosinophil

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ABSTRACT

The ultra structure of the eosinophil from normal adult camels was studied. The specific granules exhibited the basic structure of an electron-dense crystalloid core surrounded by a lighter, homogenous matrix. Unlike those of other animal species, these granules were extremely polymorphic in size and shape. Their crystalloid cores, which are generally bar-shaped and tend to fill almost the entire specific granule in most other animal species, were often segmented and demonstrated a variety of lamellated patterns that were transverse, longitudinal or concentric to the long axis of the core.

It was not uncommon to observe multiple crystalloid cores in a single granule. In addition to large specific granules, microgranules and specific microgranules were observed. The extreme polymorphism of the specific granules containing cores with a variety of lamellated patterns differentiate camel eosinophils from those of other animal species.

INTRODUCTION

During the last few years the camel immune system has generated considerable interest. This has been due to the fact that Camelids, unlike all other mammals studied so far, have a subfamily of immunoglobulin proteins that are composed of only a single polypeptide chain, rather than antibodies composed of two different polypeptide chains (Hamers-Casterman *et al.*, 1993). Although polymorpho nuclear granulocytes are generally considered part of the innate immune system, there is mounting evidence that these cells may interact so closely with the specific immune system that the

strict dichotomy of the two branches of the immune system may at times be inappropriate.

This is reflected in the findings that eosinophils interact with components of the cell-mediated immune system (Weller, 1992), since the granulocyte-microphage colony-stimulating factor has been shown to induce the expression of HLA-DR on mature eosinophils, enabling them to serve as antigen-presenting cells in stimulating T-cell responses.

Little is known about the camel eosinophil both in regards to its ultra structure as well as its function. Our preliminary observations suggest that the camel eosinophil may have unique functional properties in its ability to destroy certain parasitic pathogens. We therefore undertook an ultra structural study of the camel eosinophil as a first step in elucidating the possible unique characteristics of this cell type.

MATERIALS AND METHODS

Animals

Blood samples were obtained from the jugular veins of camels using 7 ml Vacutainers (Becton dickinson) containing Ethyllenediaminetetracetate (EDTA) as anticoagulant. These samples were from healthy adult animals maintained at the Diwan Royal Camel Farm where a strict deworming program was in effect. Eosinophil counts on this farm ranged from 1-4% of the total white blood cell count.

Sample preparation

Within thirty minutes after collection, blood samples were centrifuged at 1700 rpm for 10 minutes. After the removal of plasma, Karnovsky's fixative (2% glutaraldehyde in 2.5% paraformaldehyde in 0.2 M phosphate buffer, pH 7.38) was layered on top of the buffy coats and the tubes were refrigerated for 30 minutes. The fixative was then removed and the buffy coat layers were dissected into 2 mm x 1 mm blocks under a stereo microscope and subsequently placed in a petri dish containing fresh Karnovsky's fixative for an additional 2 hours.

One micron thick sections were stained with Toluidine blue in 1% Borax and examined by light microscopy. Representative areas that had high concentrations of eosinophils were ultra-sectioned at 60-80 nm (sliver sections) and stained by Reynold's lead citrate and uranyl acetate and examined with a Zeiss 900 electron microscope.

RESULTS

Most commonly, the nucleus of the camel eosinophil was bi-lobed. However, it was not uncommon in camels with eosinophilia to see nuclei with increased segmentation. The heterochromatin was generally distributed in the preriphery of the nucleus, whereas the loosely arranged euchromatin was found in the center. The presence of a nucleolus was extremely rare and only a few strands of rough endoplasmic reticulum were observed. Microtubules, centrioles and glycogen particles were found.

The specific granules of the camel eosinophils were extremely polymorphic, varying from round to ellipsoid or rhomboid in shape and measuring 0.4 to 2.3 micron in length and 0.3 to 1.0 micron in diameter (Figure 1). In neither healthy camels nor camels with eosinophilia did we observe any variations from the basic structure of an electron-dense crystalloid internum surrounded by an electron-lucent, homogenous matrix.

The core of the camel eosinophilic granule was extremely variable in size and shape, and often was segmented or angular (Figure 2). Occasionally, there was more than one core per granule and often the core did not fill the entire granule. The lamellations of the specific granule core were observed as longitudinal in some, and transverse to the direction of the core in other eosinphils. In some instances, both transverse and longitudinal lamellae were found in different segments of the same core. Occasionally, we observed segments of cores with lamellations suggestive of a concentric pattern. The periodicity of the core was measured as 3.5 nm.

In addition to the large specific granules, we found many microgranules (Figure 3), ranging from the 0.1 to 0.5 micron in diameter. A third type of granule, the specific microgranule, was also found. They were most commonly dumbbell-shaped (Figure 4), but they also exhibited other configurations.

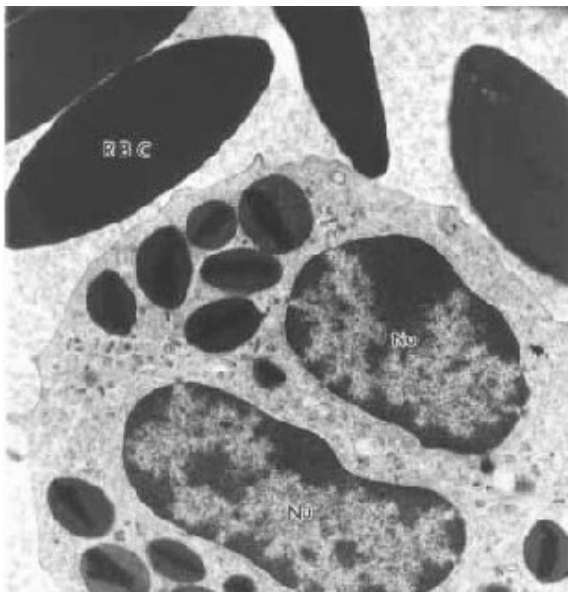

Figure 1: Electron micrograph of a camel eosinophilshowin bilobed nucleus and specific granules (x 14,000). RBC = red blood cell. Nu = nucleus, Bar = 1 micron.

Figure 2: High magnification electron micrograph showing a variety of core structures within specific granules.

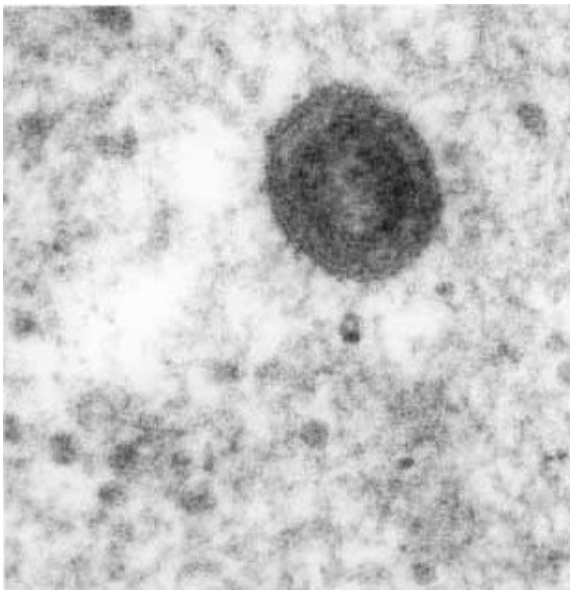

Figure 3: High magnification electron micrograph of ovoid- shaped microgranules (x 170,000).

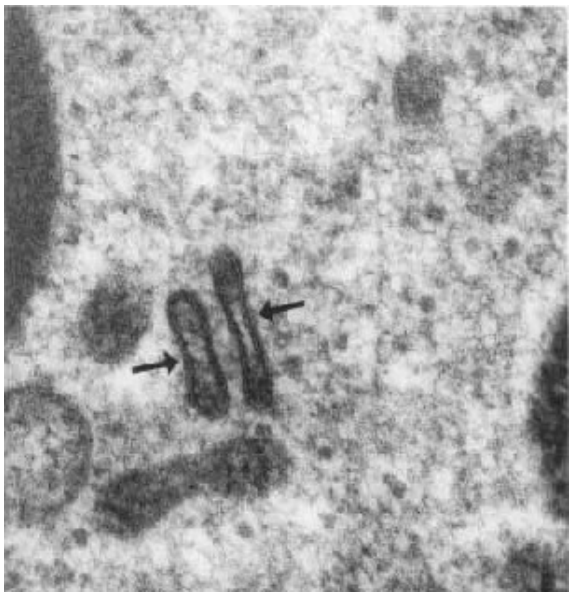

Figure 4: High magnification electron micrograph of dumbbell-shaped specific microgranules though to represent profiles of smooth endoplasmic reticulum (x 170,000).

DISCUSSION

Of interest is the extreme polymorphic nature of the camel's specific granules which is unique when compared to those of other mammals. These granules were considerably larger than those described for humans and sheep (Yamada & Sonoda, 1970; Zucker-Franklin, 1988). Their basic structure consisting of an electron-light homogenous matrix surrounding an electron-dense crystalloid core was similar to the specific granules of humans, some non-human primates, and laboratory rodents but differed from those of cows and gorillas that lack a defined core (McEwen, 1972). Sheep have been reported to have cores with and without internal cores (Yamada and Sonoda, 1970), whereas horses have been described as having homogenous specific granules (Jain, 1986) and having a number of morphological variants (Stockert *et al.*, 1993). The crystalloid core of the camel eosinophil was often variable in size and shape and was often segmented. In this respect, it differed from those of many other animal species that have cores that are central bars that generally almost fill the specific granule.

It is presently unclear which functional significance of the micro- and specific microgranules are in the camel. According to Zucker-Franklin (1988) the specific microgranules consist of smooth profile endoplasmic reticulu. It has been suggested that they may have a secretory role (Schaefer *et al.*, 1973).

The camel eosinophil appears to be uniquely characterized by extremely polymorphic specific granules with very complex internal cores. The core contains the very important arginine rich basic protein, referred to as major basic protein (Olson *et al.*, 1977; Egesten *et al.*, 1986) and is reported to be an integral part of the repertoire of the eosinophil's defense mechanisms used to inflict damage on larval helminths (Butterworth *et al.*, 1979; Wassom & Gleich, 1979; Kierszenbaum *et al.*, 1981). Future studies on the camel's eosinophil will shed clarity as to whether the complexity of the camel's specific granules is reflective of unique functional differences.

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