Short Communication

Observations on the Structures of Red Blood Cells, White Blood Cells and Platelets in Some *Felis* spp. and *Panthera* spp.

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Abstract

Blood cells and platelets from twenty-seven animals belonging to two genera of the Family Felidae, were studied using both light and transmission electron microscopes (TEM). Generally, blood cells can be categorized into red blood cells, white blood cells and platelets. The red blood cells or erythrocytes from these species were round and had neither a nucleus nor organelles. They showed a central pale area, Heinz bodies and rouleaux formation. The white blood cells could be divided into two groups; granulocytes and agranulocytes. The granulocytes (neutrophils, eosinophils and basophils) were spherical in shape with lobulated nuclei and containing specific granules in their cytoplasm. Neutrophils of the female *Felis* spp. and *Panthera* spp. showed the same nuclear appendage or Barr body. Eosinophilic granules of the *Felis* spp. were rod or oval in shape, consisted of a less electron-dense matrix and an electron-dense core or a crystalloid, with concentrically arranged lamellated structures. In contrast, eosinophilic granules of the *Panthera* spp. were round and contained electron-dense particles surrounded by a limiting membrane. Basophilic granules of *Panthera* spp. contained an electron-dense core and were surrounded by a space and a limiting membrane, while basophilic granules of *Felis* spp. consisted of an electron-dense matrix, surrounded by a limiting membrane. The agranulocytes (lymphocytes and monocytes) had a round nucleus. Generally, lymphocytes, monocytes and platelets in this study were similar to those of other common mammals.

Keywords : Blood cells, platelets, wild cat, TEM.

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บทคัดย่อ

การสำรวจโครงสร้างของเม็ดเลือดและเกล็ดเลือดในสัตว์จีนัสฟีลิสและจีนัสแพนเทอรา

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ศึกษาโครงสร้างของเซลล์เม็ดเลือดแดง เซลล์เม็ดเลือดขาวและเกล็ดเลือดในสัตว์แฟมิลี่ฟิลิดี้ จำนวน 27 ตัวจาก 2 จีนัส โดยใช้กล้องจุลทรรศน์แสงสว่างและกล้องจุลทรรศน์อิเล็กตรอนแบบลำแสงผ่าน โดยทั่วไปเซลล์เม็ดเลือดแบ่งออกเป็น เซลล์เม็ดเลือดแดง เซลล์เม็ดเลือดขาวและเกล็ดเลือด เซลล์เม็ดเลือดแดง รูปร่างกลม ไม่พบนิวเคลียสหรือออร์กาเนลใดๆ ตรงกลางเซลล์ติดสีจาง พบฮีน์ซ์บอดี้ และเรียงตัวซ้อนทับกันของเซลล์เม็ดเลือดแดง เซลล์เม็ดเลือดขาวแบ่งออกได้เป็น 2 กลุ่มคือ ชนิดมีแกรนูลและชนิดไม่มีแกรนูล เซลล์เม็ดเลือดขาวชนิดที่มีแกรนูล(นิวโทรฟิล อีโอซิโนฟิล และเบโซฟิล) มีรูปร่างกลม นิวเคลียสแบ่งออกเป็นพู ภายในไซโตพลาสมมีแกรนูลจำเพาะ นิวโทรฟิลของสัตว์จีนัสฟีลิสและจีนัสแพนเทอราของเพศเมีย พบส่วนยื่นจากนิวเคลียส เรียกว่า บาร์บอดี้ แกรนูลของอิโอซิโนฟิลของสัตว์จีนัสฟีลิส รูปร่างเป็นแท่งหรือรี ติดสีจาง และมีคริสตัลลอยด์ทึบแสงอิเล็คตรอน อยู่ภายในโดยมีการจัดตัวซ้อนเป็นชั้นๆ ในขณะที่แกรนูลของอิโอซิโนฟิลของสัตว์จีนัส แพนเทอรา รูปร่างกลม ทึบแสงอิเล็คตรอนมีเยื่อหุ้มโดยรอบ แกรนูลของเบโซฟิลของสัตว์จีนัสฟีลิสทึบแสงอิเล็คตรอน มีเยื่อหุ้มโดยรอบ ในขณะที่แกรนูลของเบโซฟิลของสัตว์จีนัสแพนเทอรา มีแกนกลางที่ทึบแสงอิเล็คตรอน มีเยื่อหุ้มหุ้มรอบ ช่องว่างขนาดใหญ่ซึ่งล้อมรอบแกนของแกรนูล เซลล์เม็ดเลือดขาวชนิดไม่มีแกรนูล (ลิมโฟไซต์ และโมโนไซต์) นิวเคลียส มีลักษณะกลม ส่วนลิมโฟไซต์ โมโนไซต์และเกล็ดเลือดในการศึกษาครั้งนี้มีลักษณะคล้ายกับสัตว์เลี้ยงลูกด้วยน้ำนม ทั่วๆไป

คำสำคัญ: เซลล์เม็ดเลือด เกล็ดเลือด แมวป่า กล้องจุลทรรศน์อิเล็กตรอนแบบลำแสงผ่าน

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Introduction

The Filidae Family consists of 3 genera; Genus Felis spp.: which includes marbled cat (*Felis marmorata*), flat-headed cat (*Felis planiceps*), jungle cat (*Felis chaus*), fishing cat (*Felis viverrina*), leopard cat (*Felis bengalensis*), and Asian golden cat (*Felis temmincki*), Genus Neofelis spp.: clouded leopard (*Neofelis nebulosa*) and Genus Panthera spp.: comprised of leopard or panther (*Panthera pardus*), tiger (*Panthera tigris*), and lion (*Panthera leo*) (for review, see Lekagul and McNeely, 1977).

The domestic feline haematology has been thoroughly documented since the 60's (Poole, 1969; Sonoda and Kobayashi, 1970[a]; Ward et al., 1972; Jain and Keeton, 1975; Presentey et al., 1980; Fondati et al., 2003). Heinz bodies occur naturally in about 10% of the erythrocytes in healthy cats because haemoglobin in the cat is relatively unstable and easily denatured. The cat eosinophil has numerous rod-shaped granules. Electron micrographs of cat basophils reveal oval membrane-bounded, specific granules, most of the lymphocytes are small and azurophilic granules are both rare and small (Brown, 1987). To date, wild feline hematology has rarely been studied. The purpose of this study was to observe

the structure of blood cells and platelets in five wild *Felis* spp. and *Panthera* spp. using both a light microscope and a TEM.

Materials and Methods

Sample collection: Twenty-seven healthy wild cats from five species *(Felis temmincki, Felis viverrina, Felis bengalens, Panthera leo and Panthera tigris*), kept at Dusit Zoo, Bangkok, Thailand, were used for this study (Table 1). Blood samples were obtained by cephalic, femoral or lateral saphenous venipuncture, during a brief period of anesthesia, induced by ketamine hydrochloride (Calypsol , Gedeon richter limited).

Light microscopic specimen preparation: Blood smears were fixed in methanol and stained with Wright-Giemsa stain for morphological evaluation of all types of blood cells.

Transmission electron microscopic specimen preparation: Blood samples were anticoagulated with EDTA and centrifuged at 2,000 rpm for 10 minutes. The plasma was removed and 2.5% glutaraldehyde in 0.1 M phosphate buffer, was layered on top of the buffy coat. After 15-30 minutes the hardened buffy coat layer was collected with a wooden dowel, placed into fresh glutaraldehyde, cut into small pieces, prior to being washed 3 times with 0.1 M PB, post-fixed with 1% osmium tetroxide in 0.1 M PB, dehydrated in graded alcohol, and embedded in Spurr's resin. Ultrathin sections were cut using a diamond knife on an ultramicrotome, placed on a mesh grid, stained with uranyl acetate and lead citrate, before examined under a TEM(JEM-200CX, JEM-2100, JEOL, Japan). Micrographs were taken at 5,000 to 20,000 magnification.

Results and Discussion

In the present study, the erythrocytes of *Felis* spp. and Panthera spp. were non-nucleated, biconcave disks and 5.0-8.4 m in diameter (n=40) (Fig. 1). In cats, horses and ruminants, a central pale area is not prominent (Thrall, 2001), however, the red cells of all the spp. in this study have a prominent central pallor, similar to that of the dog (Jain, 1993). The rouleaux formation which occurs occasionally in the dog and pig, and which is rarely seen in ruminant red cells (Brown, 1987), was common in the Felis spp. and Panthera spp. In our study, similar to that of horses and domestic cats. Heinz bodies, which in cat red cells occur naturally, were also found in the red cells of the *Felis* spp. and *Panthera* spp.

The neutrophils of *Felis* spp. and *Panthera* spp. in this study were 10.5-16 m in diameter (n=20). The nucleus often had five lobes which, in section,

Table 1 Twenty-seven healthy wild cats from five species of the Family Felidae used in this study.

Species	Female	Male
Asian golden cat (*Felis temmincki*)	1	3
Fishing cat (*Felis viverrina*)	1	1
Leopard cat (*Felis bengalensis*)	4	10
Lion (*Panthera leo*)	1	1
Tiger (*Panthera tigris*)	3	2
Total	**10**	**17**

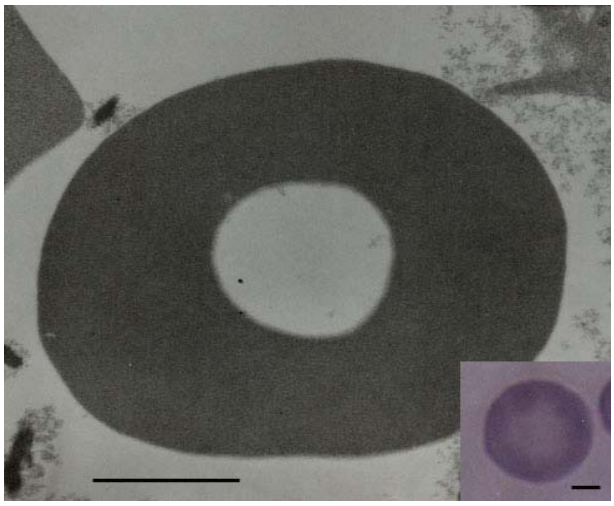

Figure 1 An electron micrograph of a red blood cell of a leopard cat (*Felis bengalensis*) shows a non-nucleated, biconcave disk. A light micrograph of a red blood cell is shown in a right bottom inset. Bars = 2 m.

Figure 2 An electron micrograph of a neutrophil of a leopard cat (*Felis bengalensis*) shows nucleus (N), mitochondria (M), specific granules (G), endoplasmic reticulum (arrow), ribosomes (R) and lysosome (Ly). A light micrograph of a neutrophil in a right bottom inset shows the Barr body. Bars = 2 m.

Figure 3 An electron micrograph of an eosinophil of a leopard cat (*Felis bengalensis*) shows nucleus (N) and specific granules (G). A left bottom inset shows the lamellae in crystalloid (arrow) in a specific granule. A light micrograph of an eosinophil is shown in a right bottom inset. Black bars = 2 m and white bar = 0.5 m.

Figure 4 An electron micrograph of an eosinophil of a lion (*Panthera leo*) shows nucleus (N) and specific granules (G). A left bottom inset shows a membrane bound specific granule (arrow). A light micrograph of an eosinophil is shown in a right bottom inset. Black bars = 2 m and white bar = 0.5 m.

Figure 5 An electron micrograph of a basophil of a leopard cat (*Felis bengalensis*): shows nucleus (N) and specific granules (G). A left bottom inset shows a membrane bound specific granule (arrow). A light micrograph of a basophil is shown in a right bottom inset. Black bars = 2 m and white bar = 0.5 m.

Figure 6 An electron micrograph of a basophil of a lion (*Panthera leo*) shows nucleus (N) and specific granules (G). A left bottom inset shows a space and membrane bound specific granule (arrow). A light micrograph of a basophil is shown in a right bottom inset. Black bars = 2 m and white bar = 0.5 m.

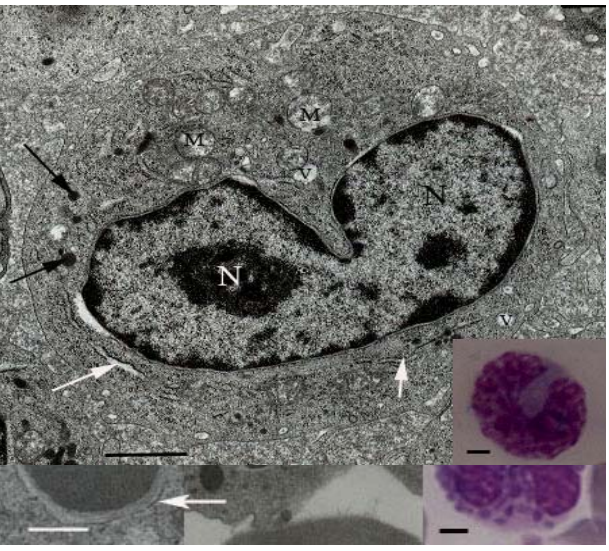

Figure 7 An electron micrograph of a lymphocyte of an Asian golden cat (*Felis temmincki*) shows nucleus (N), mitochondria (M), ribosomes (R), inclusion bodies (white arrow) and endoplasmic reticulum (black arrow). A light micrograph of a lymphocyte is shown in a right bottom inset. Bars = 2 m.

Figure 8 An electron micrograph of a monocyte of an Asian golden cat (*Felis temmincki*) shows nucleus (N), mitochondria (M), vacuole (V), vesicles (black arrow) and endoplasmic reticulum (white arrow). A light micrograph of a monocyte is shown in a right bottom inset. Bars = 2 m.

Figure 9 An electron micrograph of a platelet of an Asian golden cat (*Felis temmincki*) shows mitochondria (M), granules (G), glycogen (g) and microtubule (arrow). A light micrograph of a platelet is shown in a right bottom inset. Bars = 2 m.

could appear as separate nuclei. The chromatin was highly condensed. The granules were relatively small 0.1-0.2 m in diameter (n=10), mostly rod-shaped and less electron-dense than that of eosinophils and basophils. Neutrophils of the female *Felis* spp. and *Panthera* spp. showed the same nuclear appendage or an extra chromatin lobe resembling a drumstick. The appendage is commonly referred to as the Barr body (Fig. 2). There was no Barr body in the neutrophils of the male *Felis* spp. or the *Panthera* spp.

The eosinophils of *Felis* spp. and *Panthera* spp. in this study were 11-18 m in diameter (n=10). The ultrastructural features of specific granules in the *Felis* spp. were rod or oval shaped, 1.1-1.5 m in diameter (n=10) and consisted of a less electron-dense matrix than that of the core and had an electron-dense core or crystalloid. High-magnification electron micrographs of the crystalloid revealed concentrically arranged lamellated structures (Fig. 3) as is usually seen in domestic cats (Osaka, 1959). Eosinophilic granules of the *Panthera* spp. were mostly round, 1.0-1.2 m in diameter (n=10) and contained electron-dense particles surrounded by a limiting membrane (Fig. 4). The granules of the Felis spp., in this study, were rod shaped with prominent crystalloids, which were similar to those of humans, dogs, goats, guinea pigs, mice, rats and rhesus monkeys(Ghadially, 1988; McEwen, 1992). In contrast, the round shaped eosinophilic granules of the *Panthera* spp. in our study did not have crystalloids, and were similar to those of cows, mink and gorillas.

The basophils of the *Felis* spp. and the *Panthera* spp. in this study were 9-18 m in diameter (n=5). Ultrastructurally, the specific granules in the Felis spp. were generally round, 1.1-1.5 m in diameter (n=10) (Fig. 5). The specific granules in *Panthera* spp. were also round and 1.0-1.2 m in diameter (n=10) (Fig. 6). Electron micrographs of cat basophils (Ward et al., 1972), and basophils of the *Felis* spp. reveal a large, round homogenous matrix and electron-dense, membrane-bounded, specific granules, whereas basophils of the *Panthera* spp. reveal an electron-dense core, surrounded by space and a limiting membrane.

Small lymphocytes of the *Felis* spp. and the *Panthera* spp. in our study were 7.5-10.0 m in diameter (n=5) and had densely stained round nuclei. Small lymphocytes had small amounts of cytoplasm that stained pale blue. Large lymphocytes were 10.0-13.0 m in diameter (n=5) with more cytoplasm. Ultrastructurally, lymphocyte cytoplasm contained small numbers of mitochondria, endoplasmic reticulum, ribosomes and inclusion bodies. The nuclei were oval, with peripheral clumps of heterochromatin (Fig. 7).

The monocytes of the *Felis* spp. and the *Panthera* spp. in our study were 12-18.5 m in diameter (n=10) with a pleomorphic nucleus. The cytoplasm was relatively abundant foamy and stained

slightly gray blue. Under the electron microscope, the nucleus appeared irregular and showed dense chromatin condensation along the nuclear membrane. The cytoplasm contained mitochondria, endoplasmic reticulum, vesicles and vacuoles (Fig. 8). Monocytes were present in a relative low proportion in the peripheral blood and appear relatively similar to that of most common domestic species (Sonoda and Kobayashi, 1970[b]; Nichols et al., 1971). Ultrastructurally, monocyte cytoplasm in this study contained small vesicles, similar to those found in human monocyte cytoplasm (Tanaka and Goodman, 1972).

The platelets of the *Felis* spp. and the *Panthera* spp. in this study were round or oval, anucleated and of various sizes 2.0-5.0 m in diameter (n=10). They often clumped together. Under an electron microscopy, platelets showed a round or oval shaped mass of cytoplasm with a few thin projections or spicules extending from the surface. The cytoplasm contained several different organelles, including various granules, glycogens, mitochondria and some microtubules (Fig. 9). The platelets of these wild cats were not different from those of domestic animals. These findings can be useful for identifying the blood cells of different feline species and can assist the diagnoses of certain diseases, such as feline leukemia (Grindem, 1985), and hypereosinophilic syndrome (McEwen et al., 1985). Electron micrography is a valuable tool for studying cell cycle-related morphological changes in feline blood cells in both normal and pathological conditions. Further investigations are necessary if prompt categorization of blood cells of these species is required.

Acknowledgement

The authors thank Dr. Somchai Chotiapisitkul, Dusit Zoo, Bangkok, Thailand and the Department of Veterinary Anatomy, Faculty of Veterinary Science, Chulalongkorn University for their invaluable cooperation in this project. We also thank Assoc. Prof. Dr. Weerapong Koykul for reviewing the manuscript.

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