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Hematology, Cytochemistry and Ultrastructure of Blood Cells from Asian Elephant (*Elephas maximus*)

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ABSTRACT

Blood cells from fourteen (5 males and 9 females) adult Asian elephants were examined and cytochemical stained with Sudan Black B (SBB), peroxidase, periodic acid Schiff's reaction (PAS), a-naphthyl acetate esterase (ANAE) and b-glucuronidase (b-glu). The complete blood counts were performed using automate cell counter. There were no significant differences nearly all hematological values between the male and female elephants except the leukocyte count and fibrinogen concentration which were higher and lower, respectively, in the males than the females. Neutrophils had poorly segmented neclei and many well-differentiated granules. Neutrophils stained strongly positive to SBB, faintly stained with PAS, focal dot stained to ANAE and b-glu. Eosinophils contained 2-3 lobe nuclei, numerous small round red refractive granules with some vacuoles. Eosinophils stained moderately positive to SBB and strongly positive to ANAE but negative to b-glu. Basophils had variable number of intensely basophilic granules which did not obscure the lobed nuclei. Basophils were negative for SBB but moderately positive to ANAE and b-glu. Monocytes stained moderately positive to SBB and moderately to strongly positive to ANAE and b-glu. The bilobed cells stained moderately positive for SBB and strongly positive for ANAE and b-glu which were similar to monocytes. Ultrastructurally, they contained a large number of mitochondria similar to those of monocytes except the shape of the nuclei. The number of bilobed cells exceeded the number of the other leukocytes. Scanning electron microscopy revealed surfaces of all blood cells. Transmission electron microscopy revealed organelles within erythrocytes, platelets and all leukocytes especially bilobed cells.

Key words: Asian elephant, blood cell, cytochemistry, hematology, ultrastructure

INTRODUCTION

Asian elephants (*Elephas maximus*) are one of two species of elephant alive today. They are endangered, due to human overpopulation, diminishing and poaching (Mikota and Karn, 2000). So this endangered species in the zoo has been studied intensively to determine the health status of the individuals. Veterinary hematology serves as a screening procedure to assess general health, assess the body's ability to fight infection in adjunct to patient evaluation or diagnosis (Jain, 1993). Cytochemical method is useful in diagnosis of acute leukemia in human (Apibal, 1987). The

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purpose of the present study was to characterize the morphology, cytochemical reaction and ultrastrucrure of blood cells and hematology in the Asian elephants.

MATERIALS AND METHODS

Blood cells from fourteen (5 males and 9 females) adult Asian elephants from Khao Kheaw Open Zoo were examined and measured after staining with modified Wright and Wright's stains. The complete blood cell count was performed using the Baker 9110 (Biochem Immuno System). The cytochemical stains, including Sudan black B (SBB), peroxidase, periodic acid Schiff's reaction (PAS), a-naphthyl acetate esterase (ANAE) and b-glucuronidase (b-glu), were applied using the same methods described by Salakij *et al*. (2000).

Hematology were performed within 2 hours after blood collection. The plasma protein and fibrinogen were performed using refractometer and heat precipitation technique, respectively (Schalm *et al.,* 1975). Two direct blood smears from each elephant were stained with modified Wright and Wright's stains. A minimum of 200 leukocytes were counted for differential leukocyte determinations. EDTA blood was used to perform reticulocyte count; by staining with new methylene blue (Schalm *et al.,* 1975). The percentage of reticulocyte presented in 1,000 red blood cells (RBC) was determined. For each hematologic parameter, means, variances and standard errors were calculated using SPSS" for Window' (Norusis, 1993).

For scanning electron microscopy (SEM) and transmission electron microscopy (TEM), blood cells from 5 Asian elephants were processed as described previously (Salakij *et al.*, 2002). Identification of blood cells by SEM and TEM was based on the relative number, size, shape and distribution of granules and on nuclear appearance.

RESULTS AND DISCUSSION

There was no significant differences nearly all hematological values between the male and female elephants (Table 1) except the leukocyte count and fibrinogen concentration which were higher and lower, respectively, in the males than the females. So all hematological values were pooled (Table 1). The PCV, hemoglobin and RBC count in this study were less than those in North America, but MCV, MCH and MCHC were similar (Mikota and Karn, 2000). The PCV in both the male and female elephants in this study were slightly less than those in India (Nirmalan, *et al*., 1967) and in Sri Lanka (Silva and Kuruwita, 1993).

Red blood cells showed uniform in sized (Figures 1, 2, 3a) with 9.0 mm mean diameter. In some occasion, abnormal RBC membrane folding or echinocyte (Figure 3b), a schistocytes (Figure 3c), ruptured hole in RBC (Figure 3d) and defectived RBC with some process similar to apple stem cell (Figure 3e) were seldom detected. The red cell distribution width (RDW) in the elephant was similar to those in dogs (Jain, 1993). They were easy to form rouleaux the same degree as those in horses (Jain, 1993). The high fibrinogen (Table 1) and globulin and low albumin in the plasma (Giri *et al*., 1958), the large size of RBCs and low RBC count may all contribute to rouleaux formation easily.

Reticulocyte count in this study was zero (Figure 1f, Table 1) similar to those of Asian elephant in North America (Mikota and Karn, 2000). Ultrastructurally, RBCs contained only hemoglobin (Figure 7a).

Platelets were easily to aggregate (Figure 3f). Platelet count in this study was less than those in North America (Mikota and Karn, 2000). Anyhow, platelets/oilfield was high (Table 1) suggested that the low platelet count maybe systemic error from platelet clumps and from platelets attached to WBC (Figure 6f). Mean

platelet volume (MPV) in the Asian elephants were smaller than those in the dog and the cat (Reagan and Rebar, 1995). Ultrastructurally, platelets contained both dense granules and alpha granules (Figure 7b) which were not different from those in bovine (Fern, 2000). Some glycogen granules were also detected in some platelets similar to those in African elephant (Du Plessis and Stevens, 2002).

Neutrophils had poorly segmented nuclei and many well-differentiated granules (Figure 1a, 1c, 2a). Neutrophils stained strongly positive with SBB (Figure 2a), faintly stained with PAS (Figure 2i), focal dot stained for ANAE (Figure 2m) and b-glu (Table 3). Neutrophils of the Asian elephant were cytochemically stained different from those of Asian wild dog which were negative to ANAE (Salakij *et al*., 2000). They also different from those of Asiatic black bear which were strongly positive to ANAE (Salakij *et al*., 2005). By SEM, neutrophils were round cells with many short microvilli and some micropores (Figure 4b).

Table 1 Comparative hematology (mean ± SE) between the male and the female Asian elephants.

Hematology	Male (n = 5)	Female (n = 9)	All Asian elephant (n = 14)
PCV (%)	30.3 ± 2.3	32.6 ± 0.5	32.2 ± 0.7
Hb (g/dL)	10.8 ± 1.1	11.7 ± 0.2	11.5 ± 0.3
RBC (10^{12}/L)	2.523 ± 0.257	2.688 ± 0.0056	2.658 ± 0.080
MCV (fL)	123.1 ± 4.3	121.5 ± 1.9	121.8 ± 1.6
MCH (pg)	42.8 ± 0.5	43.5 ± 0.8	43.4 ± 0.5
MCHC (g/dL)	34.8 ± 1.0	35.8 ± 0.5	35.6 ± 0.4
RDW (%)	14.9 ± 0.4	15.3 ± 0.4	15.1 ± 0.3
Reticulocyte (%)	0 ± 0	0 ± 0	0 ± 0
WBC (10^9/L)	17.200 ± 1.086*	13.378 ± 0.636*	15.017 ± 0.821
Bands (10^9/L)	0.097 ± 0.031	0.039 ± 0.016	0.067 ± 0.018
Segmenters (10^9/L)	5.166 ± 1.016	3.905 ± 0.292	4.280 ± 0.355
Eosinophils (10^9/L)	0.742 ± 0.392	0.765 ± 0.177	0.731 ± 0.153
Basophils (10^9/L)	0.021 ± 0.021	0.004 ± 0.004	0.016 ± 0.009
Lymphocytes (10^9/L)	4.817 ± 0.779	3.661 ± 0.410	4.121 ± 0.368
Bilobed monocytes (10^9/L)	6.103 ± 0.872	4.783 ± 0.297	5.632 ± 0.514
Monocytes (10^9/L)	0.222 ± 0.120	0.187 ± 0.040	0.207 ± 0.041
Band (%)	0.6 ± 0.2	0.3 ± 0.1	0.4 ± 0.4
Segmenters (%)	30.3 ± 5.9	29.3 ± 1.7	28.8 ± 2.0
Eosinophils (%)	5.0 ± 1.5	10.7 ± 3.5	4.9 ± 1.0
Basophils (%)	4.3 ± 0.9	1.7 ± 1.2	0.1 ± 0.05
Lymphocytes (%)	27.8 ± 4.0	27.3 ± 2.8	27.3 ± 3.0
Bilobed monocytes (%)	37.4 ± 4.9	35.8 ± 2.3	37.3 ± 2.2
Monocytes (%)	1.3 ± 0.7	1.4 ± 0.3	1.3 ± 0.3
Platelet count (10^{11}/L)	3.58 ± 0.30	3.47 ± 0.27	3.52 ± 0.22
Mean platelet volume (fL)	4.1 ± 0.3	4.6 ± 0.1	4.4 ± 0.1
Platelets/oilfield	31.9 ± 5.3	23.9 ± 1.3	28.3 ± 2.7
Plasma protein (g/dL)	8.05 ± 0.29	8.06 ± 0.19	8.04 ± 0.14
Fibrinogen (mg/dL)	300.0 ± 57.7*	455.6 ± 29.4*	407.1 ± 30.5

* Significant different at $p<0.05$

Ultrastructurally, they contained two types of granules, the electron-dense granules and the paler granules (Figure 7c, 7d). The ultrastructure of basophil's granules in Asian elephant were different from those of reindeer (Henkel *et al*., 1999) and Asiatic black bear (Salakij *et al*., 2005).

Eosinophils contained 2-3 lobed nuclei, numerous small round red refractive granules with some vacuoles (Figure 1a, 2b). Eosinophils stained moderately positive with SBB (Figure 2f) similar to those in bovine, dog, horse (Raskin and Valenciano, 2000) and in reindeer (Henkel *et al*., 1999). They were strongly positive to ANAE (Figure 2n) but weak or negative to b-glu (Figure 2r). Eosinophils of the Asian elephant were cytochemically stained different from those of Asian wild dog which were negative to ANAE (Salakij *et al*., 2000). The b-glu negative in eosinophils in Asian elephant was similar to those in cat (Raskin and Valenciano, 2000) and in Asiatic black bear (Salakij *et al*., 2005). SEM examination revealed irregular surfaces of eosinophil granule contour (Figure 4a). Ultrastructurally, they contained pleomorphic bar-shaped granules, mitochondria, vacuoles and ribosomes (Figure 8a-d). The ultrastructure of eosinophil's granules in Asian elephant were different from those of reindeer (Henkel *et al*., 1999) and Asiatic black bear (Salakij *et al*., 2005).

Basophils, in Wright's stain, had variable numbers of intensely basophilic granules (Figure 1b, 1c, 1d, 1e), which did not obscure the lobed nucleus. Basophils were negative to SBB (Figure 2g) similar to those in cat, dog and horse (Raskin and Valenciano, 2000). They were moderately positive to ANAE (Figure 2o) and b-glu (Figure 2s) which different from basophils found in Asian wild dog that were very strongly positive to ANAE and b-glu (Salakij *et al*., 2000). By SEM, basophils showed smaller irregular granule contour (Figure 4e, 4f) than those of eosinophils. Ultrastructurally, they contained lobed nuclei, pleomorphic granules,

Table 2 Blood cell diameter in micrometer (mean ± SD) in Asain elephants.

Parameter	No.	Diameter
RBCs	200	9.2 ± 0.1
Bands	25	14.5 ± 0.5
Neutrophils	100	15.8 ± 0.2
Eosinophils	50	16.5 ± 0.2
Basophils	30	15.3 ± 2.0
Lymphocytes		
Small	100	8.5 ± 0.1
Medium	50	10.9 ± 0.1
Large	20	15.4 ± 0.2
Bilobed monocytes	100	14.0 ± 0.2
Monocytes	30	16.5 ± 0.2

Table 3 Cytochemical staining patterns of blood cells from 5 Asain elephants.

Cell type	SBB	PAS	ANAE	b-glu
Neutrophils	++	±	+ dot/ -	±
Eosinophils	-	-	+++	±
Basophils	-	-	++	+++
Lymphocytes	-	-	-/ focal dot/fine granular	-/ focal dot/fine granular
Bilobed monocytes	+	-	+	+
Monocytes	+	NF	+	+
Platelets	-	-	+	-
RBCs	-	-	-	-

PAS indicates periodic acid-Schiff; SBB, Sudan black B; ANAE, a-naphthyl acetate esterase; and b-glu, b-glucuronidase. Staining was score as negative (-), weak (±, fews positive cells), moderate (+), moderate to strong (++), or strong (+++). NF indicates not found.

mitochondria, vacuoles and ribosomes (Figure 9a, 9b, 9d). In some basophils, the granules were vacuolated (Figure 9c) resembling degranulation. This phenomena may caused by water solubility of basophil granules (Steffens III, 2000).

Lymphocytes were negative to SBB but have 3 patterns of reactivity for ANAE and b-glu (Figure 2q), including negative, focal dot staining and fine granular staining (Figure 2l). The cytochemical staining of lymphocytes were similar to those of Asian wild dog (Salakij *et al*., 2000) and Asiatic black bear (Salakij *et al*., 2005). By SEM, there were many type of cell surface (Figures 4c, 5a-f) which could not differentiate T-cells from B-cells. Ultrastructurally, they contained round nuclei with extensive heterochromatin, some azurophilic granules and some mitochondria (Figure 10a) which were similar to those of reindeer (Henkel *et al*., 1999) and Asiatic black bear (Salakij *et al*., 2005).

Monocytes (Figures 1c, 2d) stained moderately positive with SBB (Figure 2e) which were different from those in dog, cat, bovine and horse. Some monocytes in these animals were negative to SBB (Raskin and Valenciano, 2000). Monocytes in Asian elephant were moderately to strongly positive for ANAE (Figure 2p) and b-glu (Table 3) which were to those of Asian wild dog (Salakij *et al*., 2000) and Asiatic black bear (Salakij *et al*., 2005). By SEM, monocytes showed multiple pseudopodia attach to the other cells (Figure 4d). Ultrastructurally, they contained kidney-shaped nuclei, several mitochondria and ribosomes (Figure 10b) which were similar to those of reindeer (Henkel *et al*., 1999) and Asiatic black bear (Salakij *et al*., 2005).

The bilobed cells (Figure 1c), some of which contained trilobed nuclei (Figure 2d) were smaller than monocyte (Table 2). They stained moderately positive with SBB (Figure 2f) and were moderately to strongly positive to ANAE (Figure 2k) and b-glu (Figure 2t) similar to monocytes. The number of bilobed cells exceeded the number of lymphocyte which were different from captive Asian elephants from North America (Mikota and Karn, 2000). Ultrastructurally, they were very similar to monocytes except the shape of nuclei (Figure 10c, 10d). By SEM, it was difficult to identify bilobed monocytes from monocytes or neutrophils because of their sizes were not significant different (Table 2) so in Figure 6 (a-f) showed cell surfaces that maybe these cells.

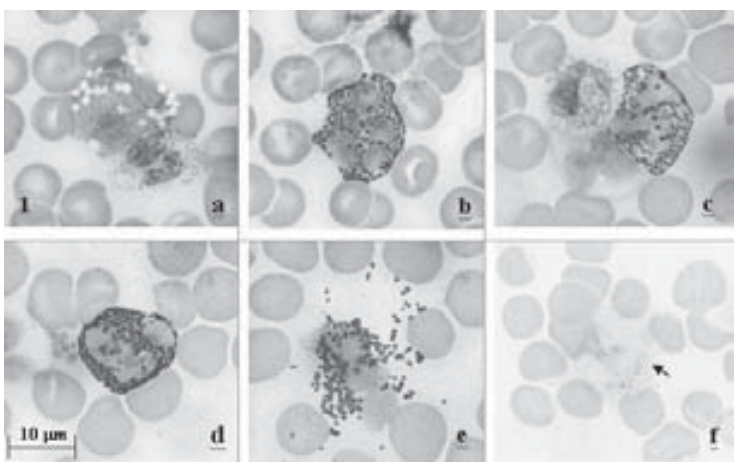

Figure 1 Blood cells in the Asian elephants in Wright's stain. **a**. A segmented neutrophil and an eosinophil with vacuoles. **b.** A basophil. **c.** A basophil, a segmented neutrophil and a bilobed monocyte. **d.** A basophil. **e.** A ruptured basophil showing shape of granules. **f.** A segmented neutrophil (arrow) in new methylene blue stain.

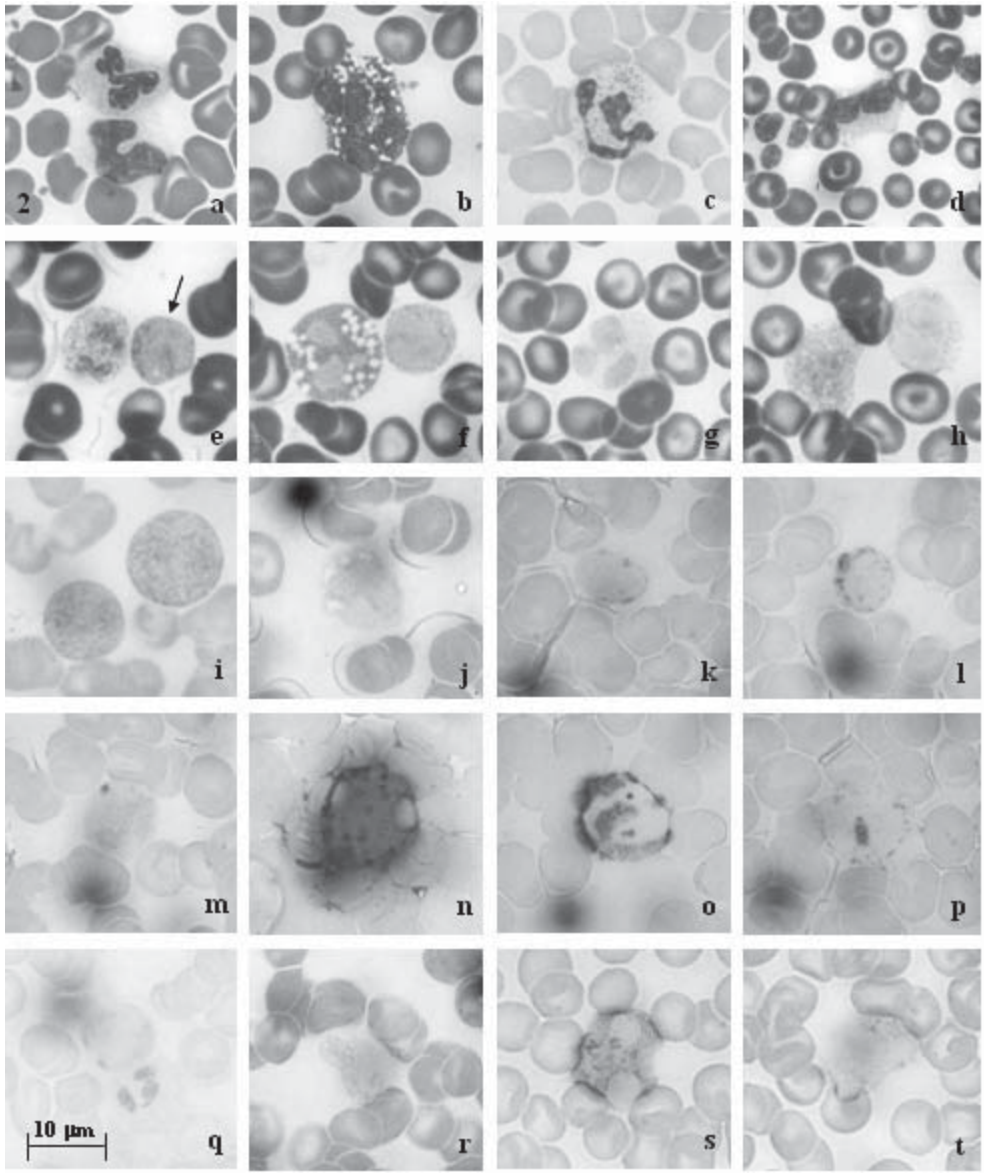

Figure 2 Cytochemical staining of blood cells compare with modified Wright stain in the Asian elephants. **a.** A monocyte and a segmented neutophil. **b.** An eosinophil **c.** A 17 µm basophil **d.** A monocyte and trilobed monocytes. **e.** Sudan Black B (SBB) positive activities in a segmented neutophil and a monocyte (arrow). **f.** SBB positive eosinophil and bilobed monocyte. **g.** Negative SBB basophil. **h.** SBB positive segmented neutophil and bilobed monocyte. **i.** PAS positive in two neutrophils. **j.** PAS negative eosinophil. **k**. ANAE positive bilobed monocyte. **l.** Fine granular ANAE reactivity in a lymphocyte. **m.** Focal dot ANAE reactivity in a neutrophil. **n.** Intense brown positive ANAE granules in an eosinophil. **o.** ANAE positive basophil. **p.** ANAE positive monocyte. **q.** β-glu positive in 13 µm lymphocyte and negative neutrophil. **r.** Weak β-glu positive eosinophil. **s.** Pink positive β-glu in granules of basophil. **t.** β-glu positive monocyte.

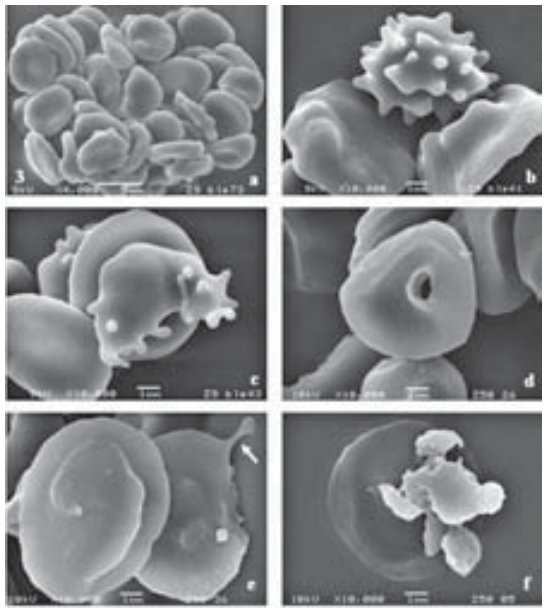

Figure 3 Scanning electron micrographs (SEM) of blood cells in the Asian elephants. **a.** RBCs showing quite uniform in size. **b.** Abnormal membrane folding and an echinocyte. **c.** A schistocyte. **d.** A ruptured hole in RBC. **e.** Defective RBC with some process (arrow) similar to apple stem cell. **f.** A cluster of platelets on an erythrocyte.

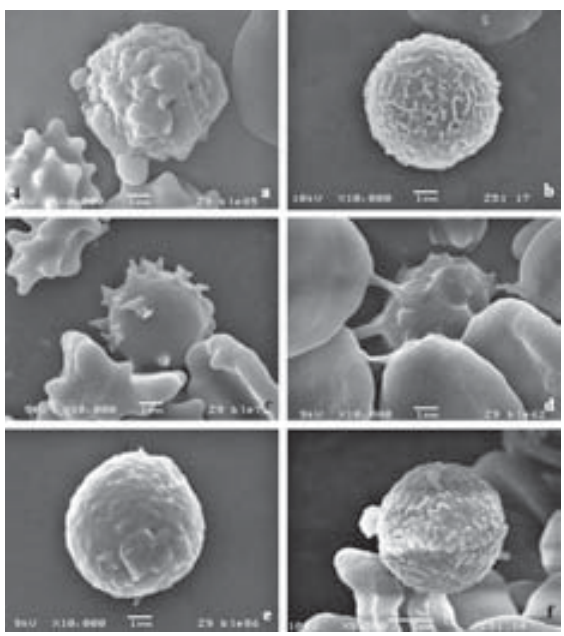

Figure 4 SEM of white blood cells in the Asian elephants. **a.** An eosinophil showing irregular granule contour. **b.** A neutrophil showing short microvilli and micropore. **c.** A lymphocyte. **d.** A monocyte showing multiple pseudopodia attach the other cells. **e.** A basophil showing small granule contour. **f.** A basophil.

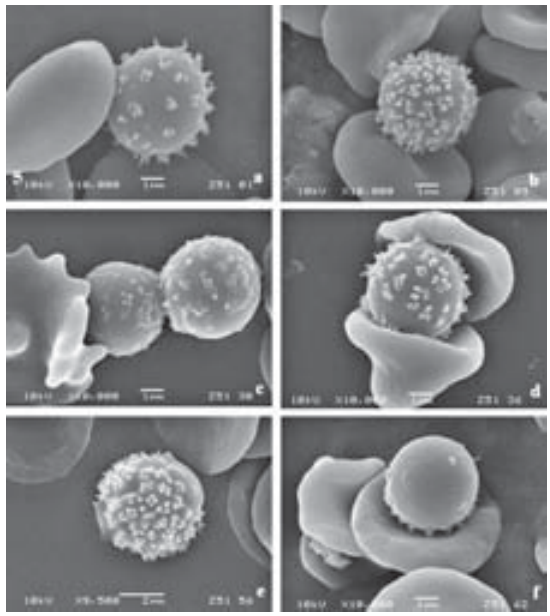

Figure 5 SEM of lymphocytes in the Asian elephants. **a.** A small lymphocyte with some membrane projections. **b.** A lymphocyte with many small membrane projection. **c.** Two lymphocytes with slightly smooth membrane. **d.** A lymphocyte between two RBCs. **e.** A lymphocyte with many small membrane projections. **f.** A lymphocyte in the concave surface of RBC.

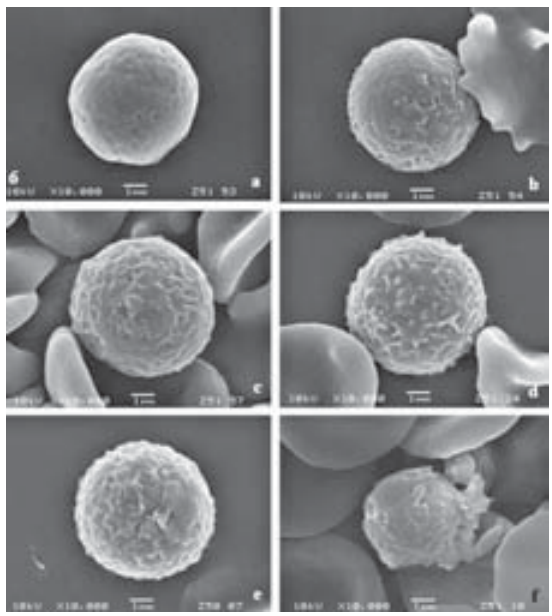

Figure 6 SEM of white blood cells in the Asian elephants. **a.** A 5. mm WBC with low membrane projections. **b.** A 5.6 mm WBC. **c.** A 6.1 mm WBC. **d.** A 6 mm WBC. **e.** A 6 mm WBC. **f.** A 4.5 mm WBC with platelet rosetting.

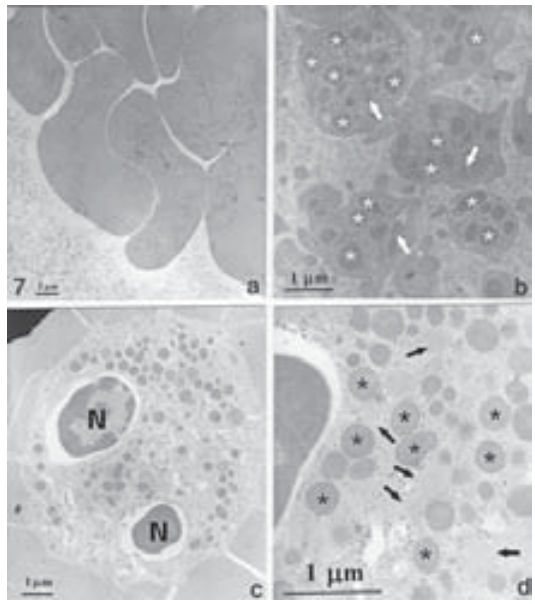

Figure 7 Transmission electron micrographs (TEM) of blood cells in the Asian elephants. **a.** A cluster of RBCs. **b.** Platelets contained dense granules (*), alpha-granules (arrows) and glycogen granules in the cytoplasm. **c.** A segmented neutrophil with two lobed nucleus (N). **d.** Higher magnification of neutrophil in (c) showing the electron dense granules (*) and the paler granules (arrows).

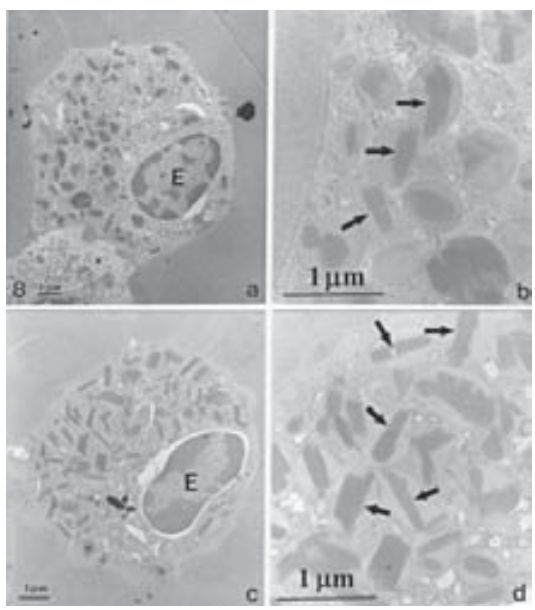

Figure 8 TEM of eosinophils in the Asian elephants. **a.** An eosinophil (E) with many granules. **b.** Higher magnification of eosinophil in (a) showing bar-shaped structures (arrows) in the granules. **c.** An eosinophil (E). **d.** Higher magnification of eosinophil in (c) showing bar-shaped structures (arrows) in the granules.

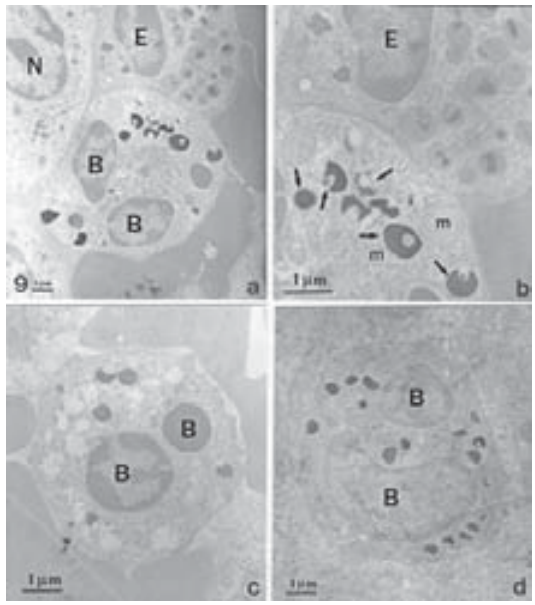

Figure 9 TEM of basophils in the Asian elephants. **a.** A basophil with two-lobed nucleus (B) next to an eosinophil (E) and a neutrophil (N). **b.** Higher magnification of basophil in (a) showing pleomorphic granules (arrows) and mitochondria in a basophil. **c.** A vacuolated basophil with two-lobed nucleus (B). **d.** A basophil (B).

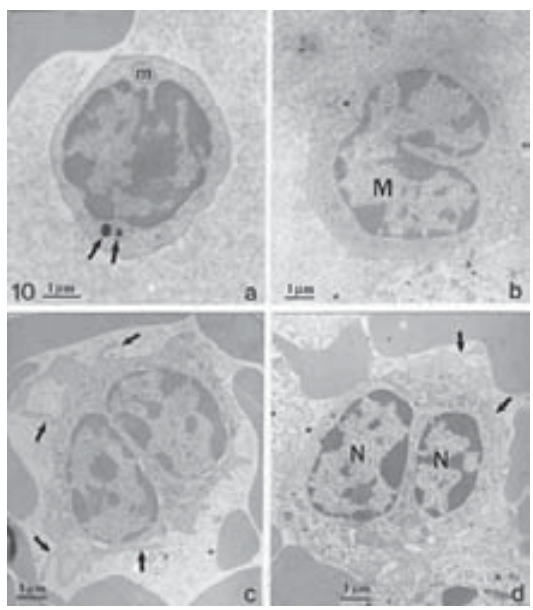

Figure 10 TEM of blood cells in the Asian elephants. **a.** A lymphocyte with mitochondria (m) and two azurophilic granules (arrows). **b.** A monocyte (M) with many ribosomes and mitochondria. **c.** A bilobed monocyte with pseudopodia (arrows). **d.** A bilobed monocyte showing bilobed nucleus (N) contining heterochromatin, many mitochondria in the cytoplasm and microvilli (arrows) on the surface of cell.

The bilobed cells were positive to SBB, ANAE and b-glu. The ultrastructure of bilobed cell were similar to those of monocytes. This study supported that they were monocytes and were the most numerous leukocytes in normal elephant. Anyhow, the number of bilobed monocytes in captive Asian elephant in North America was less than the number of neutrophils and lymphocytes (Mikota and Karn, 2000). But the Asian elephants in Sri Lanka had the number of monocyte less than only the number of lymphocyte (Silva and Kuruwita, 1993). The number of bilobed monocyte in this study was also higher than those elepants in Lampang province in the North of Thailand (Teerawat *et al*., 2000).

CONCLUSIONS

The Asian elephants have the bilobed monocytes which are different from the other mammals and characteristics of Asian elephant leukocytes. The number of bilobed monocytes exceed the number of the other leukocytes. So the bilobed monocytes are the most prevalent leukocytes in the Asian elephant. The results of this study provide more information on the morphology, cytochemical staining and ultrastructural characteristics of blood cells from Asian elephants.

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