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SHORT COMMUNICATION

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Observation of Leukocyte Differential in *Macaca fascicularis*

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Abstract
Leukocytes play a crucial role in immune defense mechanisms. The differential leukocyte count and morphological examination are commonly used in laboratory test to identify anomalies in leukocytes distribution and detect morphological irregularities. This study aimed to performed a differential leukocyte count using peripheral blood smear samples obtained from five cynomolgus monkey (*Macaca fascicularis*) bloods. Staining of peripheral blood smears was followed by observation under a light microscope to perform differential leukocyte counts and morphological examination. The differential leukocyte count results showed signs of lymphocytosis and neutropenia in samples AJ400, AB924, and AJ150, which may suggest infections, such as viral or bacterial infections. Additionally, signs of monocytosis showed in samples AB924 and AJ150, possibly attributed to bacteremia or endocarditis. However, the morphological examination showed no abnormalities or immature cells.

Keywords: differential leukocyte count, leukocyte morphology, long-tailed macaque, lymphocytosis, neutropenia.

1. Introduction

The cynomolgus monkey, also known as long-tailed macaque (*Macaca fascicularis*) is widely recognized as a preferred model animal in biomedical research due to the similiarity of its anatomical, physiological, genetic, and immunological similiarities to human. *M. fascicularis* is commonly used in studies to advance human health (Hakim *et al.* 2022). They are well known as a good models for vaccine and drug development (Cauvin *et al.* 2015; Hakim *et al.* 2022). However, using *M. fascicularis* as model animal also has disadvantage. Close contact between researchers and these macaques may pose a risk of zoonotic disease transmission (Rosyid *et al.* 2023).

The differential leukocyte count is a commonly utilized laboratory test for detecting anomalies in the percentage distribution of various leukocytes types and identifying morphological irregularities (Bajimaya *et al.* 2021). Leukocyte, often referred to as white blood cells, is one of several components in the blood with larger size compared to erythrocytes, but present in fewer numbers. Leukocytes are divided into two main groups, namely agranular and granular. Agranulocytes consist of lymphocytes and monocytes, while granulocytes consist of basophils, eosinophils, and neutrophils (Niagita and Mardina 2019).

These blood cells play a crucial role in the body's defense against infections, functioning through mechanisms like phagocytosis and antibody production. This represents the response leukocytes initiate when the body is under attack by infectious agents. Each type of leukocytes has its own function. For example, monocytes digest damaged cells, and lymphocytes which protect body against virus-infected cells and tumor cells. Basophils release histamine during allergic reactions, while eosinophils defend against viral infections and contribute to tissue damage and inflammation as observed in various diseases. Neutrophils are highly effective in digesting pathogens (Latimer and Duncan 2011; Saidani *et al.* 2024). Fluctuations in the percentage of leukocyte differentials may reflect the individual's health status (Purnomo *et al.* 2015).

The differential white blood cells (WBCs) count can be performed using a peripheral blood smear. Typically, five fully developed WBCs are examined and evaluated in the peripheral blood smear to assess the percentage of leukocyte differentials, detect the presence of immature and irregular cells, and identify any morphological abnormalities (Al-Qudah and Suen 2021).

Peripheral blood smear need to be stain to facilitate examination under the microscope, often utilizing techniques like the Romanoswky staining technique, which encompasses various staining methods such as Wright staining, Giemsa staining, and Diff-Quik. Among these methods, Diff-Quik stands out as one of the commonly used commercial staining agents for blood smear staining (Cowell *et al.* 1999).

2. Materials and Methods

2.1 Making Peripheral Blood Smear Preparation

Blood samples were collected from five *M. fascicularis* sourced from Primate Research Center-IPB University. A drop of blood is dispensed onto one end of a glass slide, and then another slide is placed at a 30-40° angle over the blood drop. Subsequently, the second slide is swiftly push to the other end, creating a thin layer of blood, followed by air-drying before entering the staining phase. Diff-Quik staining is perform by using the Diff-Quick™ Staining Kit.

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Fixation is first done by using Diff-Quick Fix with five dips followed by staining is using Diff-Quick I (Eosin), and then dipping into Diff-Quick II (Methylene blue) solution with five dips each then rinsed with running water, and allowed to dry (Cowell *et al.* 1999). Blood smear will be used for differential leukocyte count and leukocyte morphology examination.

2.2 Differential Leukocyte Count

The differential leukocyte count will be conducted by using peripheral blood smear under light microscope with a maginification of 100×. Leukocytes grouped into each type of leukocyte: basophils, eosinophils, neutrophils, lymphocytes, and monocytes counted by oberserving every 100 leukocytes using an analogue cell counter.

2.3 Leukocyte Morphology Examination

Peripheral blood smear will be observed under light microscope with a maginification of 100× to assess leukocytes morphologically, detecting any abnormalities and the presence of immature or irregular cells.

3. Results

3.1 Differential Leukocyte Count

Leukocyte differentiation is conducted using blood samples obtained from *M. fascicularis*. The normal differential leukocyte percentages in Table 1 are used as a comparison to evaluate the leukocyte differential percentages in samples AJ405, AJ400, 151024, AB924, and AJ150 in Table 2.

The differential leukocyte count results in Table 2 indicate that basophils and eosinophils in all samples are within the normal percentage range, according to the reference in Table 1. Samples AJ4025 and 151024 have neutrophils, lymphocytes, and monocytes within the normal range. However, samples AJ400, AB924, and AJ150 show a decreased percentage of neutrophils and an increased percentage of lymphocytes. Furthermore, samples AB924 and AJ150 exhibit an increased percentage in monocytes.

Table 1. Reference range of normal leukocyte differential percentages in *Macaca fascicularis* (Park *et al.* 2016).

Leukocyte type	Male (%)	Female (%)
Basophil	0.3 ± 0.2	0.3 ± 0.2
Eosinophil	0.6 ± 0.6	0.7 ± 0.6
Neutrophil	48.4 ± 16.4	50.7 ± 14.6
Lymphocyte	47.5 ± 15.6	45.2 ± 14.0
Monocyte	2.7 ± 1.1	2.5 ± 0.8

Table 2. Leukocyte differential counts for AJ405, AJ400, 151024, AB924, and AJ150 results.

Leukocyte type	Sample name				
	AJ405♂	AJ400♂	151024♂	AB924♀	AJ150♂
Basophil (%)	0	0	0	0	0
Eosinophil (%)	0	0	0	0	0
Neutrophil (%)	57	6	44	23	10
Lymphocyte (%)	41	92	52	72	81
Monocyte (%)	2	2	4	5	9

3.2 Leukocyte Morphology

The morphology of leukocytes in the blood smears of *M. fascicularis* samples was observed at a maginification of 100×, as shown in Figure 1 and Figure 2.

4. Discussions

Differential leukocyte count results shown in the Table 2 reveal that across all samples, no basophils and eosinophils were detected, aligning

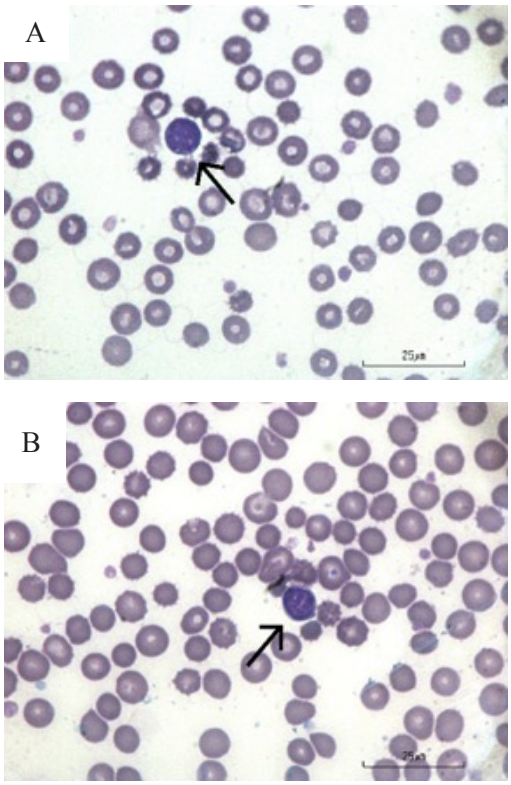

Figure 1. Morphology lymphocyte in sample AJ400 (A) and sample AJ150 (B) at a magnification of 100×, arrows indicate the presence of lymphocytes.

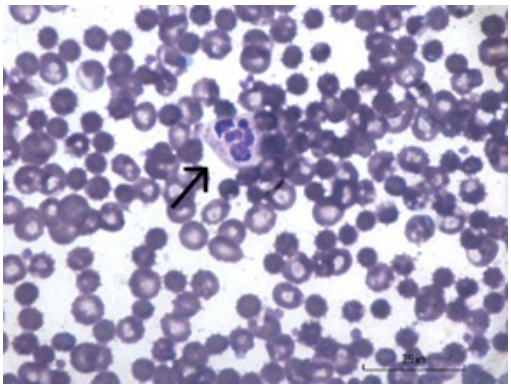

Figure 2. Morphology neutrophil in sample AJ400 at a maginification of 100×, arrows indicate the presence of neutrophils.

with the normal percentage range for basophils and eosinophils indicated in Table 1. Basophils, involved in allergic reactions and inflammation, and eosinophils, providing protection against parasites like worms, were absent in the samples, indication no allergic reactions or parasitic infections (Latimer and Duncan 2011).

In samples AJ405 and 151024, the percentages of neutrophils, lymphocytes, and monocytes are within the normal range. Neutrophils act as the first line of defense in our immune system (Erianto *et al*. 2020). Meanwhile, lymphocytes play crucial role in adaptive immune response, and monocytes contribute to the nonspecific (innate) immune system through phagocytosis (Alam and Gorska 2003; Chiu and Bharat 2016). These results indicate that the leukocyte differentials in AJ405 and 151024 show no specific response to infection, suggesting proper functioning of the immune system.

However, in samples AJ400, AB924, and AJ150, there is a notable decrease in the percentage of neutrophils (neutropenia) and an increase in the percentage of lymphocytes (lymphocytosis) above normal levels, particularly evident in sample AJ400 where there's a significant rise in lymphocyte percentage. This could be attributed to viral or bacterial infection and may also be influenced by various factors such as environmental conditions, nutritional intake, age, and individual stress levels (Purnomo *et al.* 2015). Additionally, samples AB924 and AJ150 exhibit a slight increase in the percentage of monocytes (monocytosis), although not significant. Monocytosis may arise due to conditions like bacteremia and endocarditis, also, monocytosis may be observed in both acute and chronic stages of disease (Latimer and Duncan 2011).

Furthermore, the examination of leukocyte morphology shown in Figure 1 and Figure 2. In Figure 1, lymphocytes in image A exhibit a round morphology with a large nucleus filling the entire cell, obscuring the cytoplasm, while lymphocytes in image B have a morphology where the nucleus nearly fills the cell, and both lymphocytes lack granules. In Figure 2, neutrophils are observed to have a round morphology with granular cytoplasm and segmented nuclei. This examination reveals no abnormalities or immature cells.

Limitations of the differential leukocyte count are time consuming, reliance on manual counting and morphological assessment that can lead to error or bias result (Kim *et al.* 2014). For future research, it is recommended to conduct further analysis of the examination results. Additionally, expanding the variety samples across different health conditions could provide a broader understanding of leukocyte morphology under various physiological and pathological states.

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