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Flow cytometric characterization of T lymphocyte subsets in the peripheral blood of Chinese rhesus macaques: Normal range, age- and sex-related differences

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Abstract

Available data on the normal levels of white blood cell populations in healthy rhesus macaques (*Macaca mulatta*) originated and living in China is scanty. To obtain such data, blood samples from 150 Chinese rhesus macaques were collected and the normal range of white blood cells and their subsets were analyzed according to age and sex by flow cytometry. CBC data showed that the count of total white blood cells and lymphocytes decreased with age. Phenotypic analysis of CD4 and CD8 expression on CD3+ T lymphocytes showed that the percentage of CD4+ T cells (51.4 ± 9.6%), CD4−CD8− T cells (8.5 ± 4.1%) and the ratio of CD4+ T to CD8+ T cells (1.26 ± 0.55) decreased with age; and the percentage of CD8+ T cells (42.0 ± 9.7%), CD4+CD8+ T cells (1.3 ± 0.9%) and CD3+ lymphocytes (55.3 ± 13.3%) increased with age. However, no statistically significant difference was observed between the male and female groups in most parameters in these monkeys except for the percentage of CD4+CD8+ T cells. This study provided basic information about blood cell count and T lymphocyte subsets in Chinese rhesus macaques. It may be useful for comparative studies using Indian and Chinese rhesus macaques.

Keywords: Chinese rhesus macaques; Lymphocyte subpopulations; Age difference; Sex difference

1. Introduction

Rhesus macaques (*Macaca mulatta*) have been used in various studies on human diseases, especially on infectious diseases such as AIDS (Letvin et al., 1985; Miller et al., 1989; Kestler et al., 1990; Palca, 1990; Joag et al., 1997; Joag, 2000; Alexander et al., 1999) and in vaccine research and development (Lamb-Wharton et al., 1997; Wilson et al., 2006). Most of the rhesus macaques used in the medical biology field worldwide were of India origin and the China origin rhesus macaques (Chinese rhesus macaques) had only been used extensively in recent years in order to resolve the shortage of India origin rhesus macaques (Roberts et al., 2000). However, differences have been observed in the host responses to pathogen infections between Chinese and Indian rhesus macaques (Trichel et al., 2002; Ling et al., 2002a,b; Reimann et al., 2005). We do not know whether the differences between Chinese and Indian rhesus macaques will hinder its usefulness and the comparability of experimental data generated from these rhesus macaques. The researches into the immunological background of Chinese rhesus maca-

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ques will provide us with more information about these monkeys, and take us closer to the answer.

In addition to the difference in their habitat or geographical origin, there are still general biologic differences between the Chinese and the Indian rhesus macaques, such as their morphometrics (Clarke and O'Neil, 1999), behavioral and physiological characteristics (Champoux et al., 1997), mitochondrial DNA and genetic background (Smith and McDonough, 2005; Smith, 2005). Differences in immune responses to pathogen infections indicated that the immune system, which is closely related to the containment of infectious diseases by the host, might be different between the two geographical populations. Although a lot has been known about the immune system of the Indian rhesus macaques, little has been done to characterize the immune system of the Chinese rhesus macaques, especially those living in China (Yang et al., 2007).

The complete blood count (CBC) is a routine hematology laboratory test with demonstrated clinical usefulness, and provides important basic information about the host immune system. CD4+ T lymphocyte count is an important parameter in HIV/AIDS clinical tests and related experimental researches. In an effort to provide such basic information about the normal immune system of Chinese origin rhesus macaques, we obtained blood samples from 150 rhesus macaques originated and living in China. Complete blood count (CBC) and the size of lymphocyte subsets were examined using an automated haemacytometer and flow cytometry. The normal range of CBC, lymphocyte subsets and the influence of age and sex on these parameters were reported in this paper.

2. Materials and methods

2.1. Blood samples

Samples of peripheral blood were obtained from 150 healthy Chinese rhesus macaques housed in a monkey farm near Beijing. The basic information about these monkeys is shown in Table 1: they are about 1–18 years old (mean = 5.64 years), 90 of them are males, and 60 females. There is no statistical difference in the age distribution between the males and females.

All the blood samples were drawn into vacuum EDTA tubes and CBC tests were performed immediately after the samples were brought into the laboratory. Briefly, 50 μl whole blood from each blood sample was put into a 1.5 ml eppendorf tube (Axygen, USA) and then the data were collected using an automated haemacytometer (MEK-6318K, Nihon Kohden, Japan).

2.2. Flow cytometry

To prepare the samples for flow cytometry, whole blood was stained with fluorescent dye labeled monoclonal antibodies as described previously (Yang et al., 2006, 2007; Qiu et al., 2008). Briefly, 100 μl of whole blood from each sample was added to each 12 mm × 75 mm polystyrene test tube (Falcone, Lincoln Park, NJ, USA) containing pre-added monoclonal antibodies against CD3, CD4 and CD8 (CD3− FITC, clone SP-34; CD4-PerCP, clone L200; and CD8-PE, clone RPA-T8, all from BD Pharmingen, San Diego, USA) and incubated for 20 min at room temperature in the dark. Red blood cells were lysed with FACS lysing solution (Becton Dickinson, USA) following the manufacturer's instruction. The samples were washed thoroughly in 1× phosphate-buffer saline (PBS) by centrifugation; then cell sediments were suspended in 1× PBS.

When all the samples were properly stained, cells were collected with a 4 color flow cytometer, FACSCalibur (Becton Dickinson), which was equipped with a 488 nm argon ion laser and a 635 nm red diode laser. CD3+ cells from lymphocytes that were gated on forward scatter versus side scatter dot plot were used to analyze CD4+ and CD8+ lymphocyte subsets using Cellquest-pro software (Becton Dickinson, CA, USA).

Table 1
Age, sex and body weight of the monkeys of which the blood samples were collected

Age groups (years)	Males			Females		
	N	Age (years)[a]	Weight (kg)[a]	*N*	Age (years)[a]	Weight (kg)[a]
1∼	57	3.1 ± 1.2	4.1 ± 1.7	43	2.8 ± 1.2	3.5 ± 1.0
6∼	15	7.7 ± 1.5	7.7 ± 1.0	10	7.8 ± 2.0	6.3 ± 1.3
11∼	14	13.1 ± 1.4	9.4 ± 1.1	4	12.3 ± 1.9	6.4 ± 1.0
16∼	4	17.0 ± 0.8	9.8 ± 1.3	3	17.7 ± 0.6	7.5 ± 0.9
Total	90	6.0 ± 4.6	5.8 ± 2.7	60	5.0 ± 4.3	4.3 ± 1.7

[a] Mean ± (S.D.).