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Correlating Changes in Body Weights and Immune System Parameters in Cynomolgus Macaques

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ABSTRACT

With the continued increase in the development of biological and immune-modulating therapeutics, there is a need to assess for potential toxicity of the immune system, especially in relation to other toxicity parameters. Assessment of the immune system is routinely achieved through the addition of endpoints, such as immunophenotyping of circulating cells. Due to the nature of these therapeutics, the cynomolgus macaque is often the most appropriate test system to use. A new development in the recent past is that the body weights of cynomolgus monkeys available for preclinical studies have decreased due to the high demand and use of animals in research. To improve the assessment of the drug-related impact on the immune system, the typical phenotype of circulating immune cells was established in both young adult and mature test article-naïve cynomolgus macaques. Blood was collected while animals were conscious and restrained. The following cell populations: T cells, Helper T cells, Cytotoxic T cells, Total B cells, NK cells, and monocytes were quantified using BD FACSCanto II flow cytometer. Linear regression was performed to determine if there was a correlation between animal body weight and the circulating immune cell populations. The definitive analysis revealed that there was no correlation between body weight and the relative cell populations in young and mature animals. Furthermore, there was no difference in the relative cell population counts when comparing young adult and mature animals.

INTRODUCTION

For preclinical studies, the age range is one of the critical parameters for animal selection. In general toxicology studies, the age range for a young adult is 2 to 4 years old. When there is a need for preclinical testing in an older population, mature cynomolgus monkeys of over 4 years are selected. In recent years, young adults enrolled in preclinical toxicology studies are closer to the 2-to-3-year range, and consequently, the body weights of available animals have decreased. The objective of this assessment was to determine if there was a correlation between animal body weight and baseline circulating immune cell populations (absolute) using two age populations.

MATERIALS AND METHODS

A total of 142 young adults and 132 mature test article-naïve cynomolgus monkeys were selected for this evaluation.

Primary enclosure complied with the Animal Welfare Act and recommendations set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). Animals were housed in a temperature- and humidity-controlled environment and a 12-hour light/dark cycle. PMI LabDiet® Fiber-Plus® Monkey Diet 5049 biscuits and water was provided ad libitum. Data collected during the acclimation, such as body weight, clinical observations, and clinical pathology parameters, were reviewed, and all animals were considered suitable for use on study and in good health.

Assessments of the selected circulating immune cells indicated no differences between males and females within the same age range (data not presented) and therefore, for the purpose of this evaluation, males and females were combined.

Table 1. Selected Animal Population

Category		Number of Animals	Age Range	Bodyweight at Dosing Initiation
Young Adult	Males	71	2.1 to 3.02 years	Average = 1.9 kg (1.7 to 2.4 kg)
	Females	67		Average = 1.9 kg (1.6 to 3.1 kg)
Mature	Males	66	4.3 to 9.7 years	Average = 6.0 kg (4.4 to 8.6 kg)
	Females	66		Average = 4.0 kg (2.7 to 7.2 kg)

Blood sample for immunophenotyping was collected from awake, restrained animals, into dipotassium ethylenediaminetetraacetic acid (K_2EDTA). Flow cytometry analysis was performed on fresh whole blood, using BD FACSCanto II flow cytometer.

Table 2. Phenotype of Circulating Immune Cell Populations

Population Cell Type	Population Phenotype	Parent Population (Gate)
Total T cells	CD3+	Lymphocyte
T Helper Cells	CD3+CD4+CD8-	CD3+ (Lymphocyte grandparent)
T Cytotoxic cells	CD3+CD4-CD8+	CD3+ (Lymphocyte grandparent)
Total B cells	CD3-CD20+	Lymphocyte
Natural Killer (NK) cells	CD3-CD16+	Lymphocyte
Monocytes	CD3-CD14+	Monocyte

Statistical analysis using linear regression, modeling the relationship between circulating immune cells and body weights, was applied to the data for the respective age range. The linear relationship between two variables is positive when both increase together or negative when one increases as the other decreases, in which cases there would be a direct relationship. A value of zero (0) indicates that there is no linear relationship between the two variables evaluated.

RESULTS

Young Adult - Sex Combined (Age Range 2 to 3 Years Old)—Acclimation Period

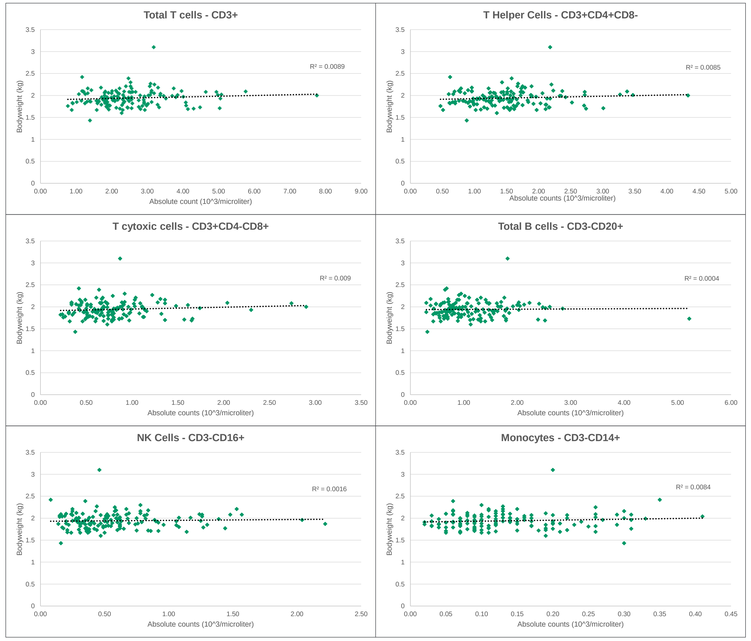

Figure 1. Linear Regression Absolute Counts on Respective Circulating Immune Cells—Young Adult Animals

Graphs generated indicated no variation in baseline absolute count values in mature animals compared to young adults. The linear regression applied to respective cell populations indicated no correlation with body weights.

Table 3. R Values per Cell Population

Population Cell Type	Young Adult - R^2 Value	Mature - R^2 Value
Total T cells	0.0089	0.0036
T Helper Cells	0.0085	0.0034
T Cytotoxic cells	0.009	0.0074
Total B cells	0.0004	0.013
Natural Killer (NK) cells	0.0016	0.0002
Monocytes	0.0084	0.0951

CONCLUSIONS

Based on calculated R^2 values equal to zero for all cell populations evaluated, it was confirmed that there was no correlation between body weights and baseline absolute cell population counts for T cells, Helper T cells, Cytotoxic T cells, Total B cells, NK cells, and monocytes, in smaller/younger animals currently available for safety assessment on general toxicology studies and heavier/older animals. This confirms that the assessment of the drug-related impact on the immune system can reliably be performed using currently available cynomolgus macaques in the age range of 2-to-3 years old with an average body weight of 1.9 kg.

Mature - Sex Combined (> 4 Years Old)—Acclimation Period

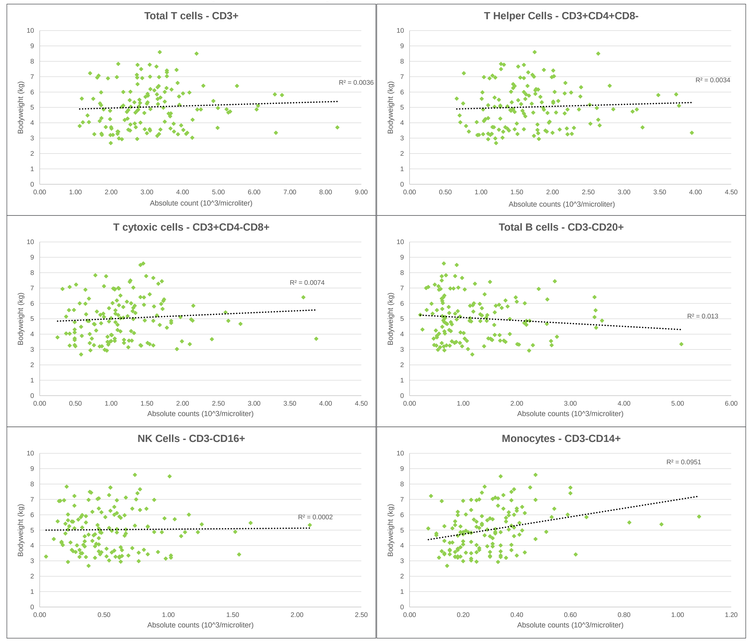

Figure 2. Linear Regression Absolute Counts on Respective Circulating Immune Cells—Mature animals

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