Establishment of Preclinical Immunotoxicity Reference Based on a Large-scale Immunophenotyping Data in Cynomolgus Monkey

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Background and Purpose

Cancer immunotherapy has rapidly emerged in the past few years. The success of immunotherapy relies on the immune system. Therapeutics modulating the immune system could sometimes become double-edged swords resulting in collateral damage to normal tissues. Severe and even fatal adverse effects have been reported during clinical trials of immunomodulatory therapeutics, highlighting the need for assessing immunotoxicity in preclinical studies.

One preclinical immunotoxicity solution recommended is immunophenotyping to identify the specific cell populations affected and provide useful clinical biomarkers. In our lab, we established a reliable lymphocyte immunophenotyping reference range in cynomolgus monkey, which can aid in the immunotoxicity assessment in preclinical study.

Methods and Test System

Animals
Cynomolgus monkeys used in this report were from China. All animals were naïve and maintained in compliance with the Guide for the Care and Use of Laboratory Animals. All animal research was approved by the Institutional Animal Care and Use Committee.

Immunophenotyping procedures
Blood samples were collected with K_2EDTA and washed with 2 mL of DPBS by centrifugation for five minutes at 400 g. Antibody cocktail (FITC anti-NHP CD45, V450 anti-human CD3, PerCP-Cy5.5 anti-human CD4, PE anti-human CD8, APC anti-human CD20, and BV 510 anti-human CD16) was incubated with each sample at room temperature for 20 to 30 minutes, followed by the wash and re-suspend with DPBS, and analyzed using BD LSRFortessa™ flow cytometer with FACS Diva software.

Results

The immunophenotyping was conducted using blood collected from 560 naïve male and 578 naïve female animals. The percentage of lymphocytes was reported in Table 1, including total T cells (CD3+), helper T cells (CD3+ CD4+ CD8-), cytotoxic T cells (CD3+ CD4- CD8+), B cell (CD3- CD20+), and natural killer (NK) cell (CD3- CD16+).

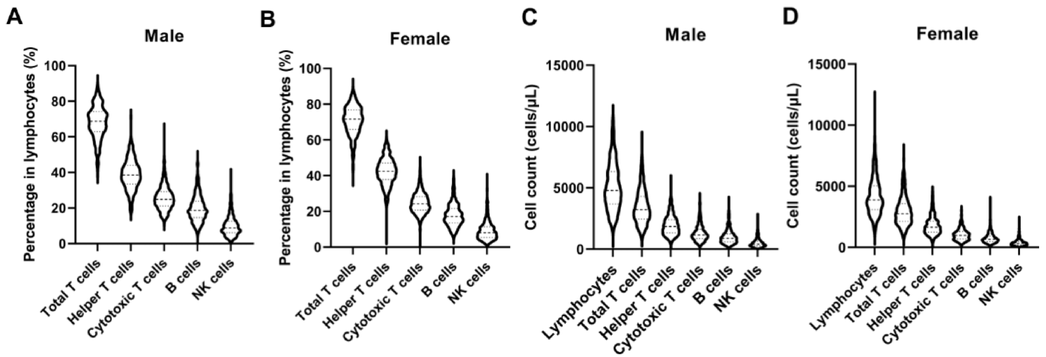

Figure 1. The distribution of the percentage (A, B) and cell count (C, D) of lymphocyte subpopulations in male and female cynomolgus monkeys.

To assess the distribution of the data, a variety of parameters were calculated as the summary in Table 1. The distribution of lymphocytes can also be visualized with the violin plots in Figures 1 A and B.

Besides, the absolute count of lymphocytes from hematology analysis is used to calculate the cell count of lymphocyte subpopulations. The distribution of the data is illustrated in Figures 1 C and D, and the distribution is summarized in Table 2.

Table 1. Range for the percentage of lymphocyte subpopulations

Parameter	Total T cells		Helper T cells		Cytotoxic T cells		B cells		NK cells	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean	68.2	70.9	38.8	42.3	25.5	24.7	19.6	17.8	9.8	9.2
Std. Deviation	9.2	8.1	8.4	7.5	6.8	5.7	7.4	6.2	5.5	5.3
Minimum	38.6	38.6	17.4	5.4	10.8	2.9	2.5	5.2	0.5	0.3
2.5% Percentile	**47.3**	**51.8**	**21.8**	**26.6**	**14.4**	**14.9**	**7.9**	**7.6**	**2.3**	**2.4**
10% Percentile	55.8	60.9	28.4	32.4	17.5	17.9	11.1	10.4	3.8	3.8
25% Percentile	62.9	65.8	33.4	37.9	21.1	20.7	14.6	13.6	6.1	5.4
Median	68.8	71.6	38.6	42.5	24.9	24.2	18.6	17.1	8.8	8.0
75% Percentile	74.3	76.8	43.9	47.1	29.2	28.3	23.8	21.6	12.5	11.5
90% Percentile	80.2	80.6	49.8	52.5	34.1	32.0	29.8	25.4	17.4	15.9
97.5% Percentile	**84.5**	**84.3**	**55.4**	**56.7**	**41.0**	**37.4**	**36.8**	**33.2**	**24.1**	**24.2**
Maximum	90.1	89.8	71.1	61.5	64.3	47.4	48.4	39.7	39.3	38.6
Number	560	578	560	578	560	578	560	578	560	578

Data was presented as a percentage of total lymphocytes.

Table 2. Range for the absolute cell count of lymphocyte subpopulations

Parameter	Lymphocyte		Total T cells		Helper T cells		Cytotoxic T cells		B cells		NK cells	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean	5072	4189	3453	2980	1942	1769	1312	1045	1006	747	496	373
Std. Deviation	1878	1559	1360	1187	785	732	661	483	576	406	348	251
Minimum	1190	995	953	751	483	165	306	90	140	139	27	12
2.5% Percentile	**2062**	**1795**	**1349**	**1196**	**748**	**705**	**403**	**335**	**276**	**236**	**88**	**75**
10% Percentile	2803	2460	1858	1716	1025	974	609	503	414	355	157	137
25% Percentile	3683	3088	2461	2127	1333	1246	870	676	602	468	261	207
Median	4785	3885	3225	2761	1844	1639	1158	979	886	653	401	321
75% Percentile	6325	5030	4312	3582	2406	2145	1626	1294	1244	926	630	463
90% Percentile	7747	6391	5387	4658	3026	2816	2213	1699	1785	1265	951	656
97.5% Percentile	**9359**	**7874**	**6571**	**5873**	**3770**	**3546**	**2952**	**2193**	**2629**	**1728**	**1415**	**1053**
Maximum	10760	11980	8846	7853	5607	4607	4255	3114	4009	3926	2721	2397
Number	560	578	560	578	560	578	560	578	560	578	560	578

Data was presented as number of cells per microliter.

Conclusions

Based on the immunophenotyping data from 1138 naïve animals, a reference range was established for the percentage and absolute count of lymphocytes in cynomolgus monkeys, which could serve as a reliable reference to interpret the preclinical immunotoxicity findings.

References

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