Historical Immunophenotyping Data in Pre-clinical Species

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1 ABSTRACT

Flow cytometry allows multiparametric analysis of thousands of particles per second and helps to adequately identify or functionally characterize complex cell populations of interest. It is often used in basic research, discovery, preclinical and clinical trials. During the preclinical phase development of new drugs, flow cytometry has routinely been used for assessing the immunotoxic effects of a candidate drug by evaluating the immunophenotype of various cell populations in whole blood, tissues, or other matrices. Despite the fact that it is being used by multiple different laboratories, combined database presentation and comparison of preclinical immunophenotyping data in various animals, strains and tissues/whole blood has been limited. The objective of this study was to compile relevant preclinical immunophenotyping data from monkeys (cynomolgus, rhesus), dogs (beagle), rats (Sprague-Dawley, Wistar, Fisher) and mice (various strains), from different matrices. The database from a single site was extracted from control data generated from over fifteen years of work. Specie, strain, age, cell populations and sample types were grouped to provide a representation of expected cell population distribution profiles.

2 METHODS

Whole blood samples were collected in K_2EDTA tubes, while thymus, spleen, lymph nodes and bone marrow were collected and processed to single cell suspension. An appropriate volume of the cell suspensions were further used for staining using the specie specific antibodies listed below.

Cell populations	NHP markers	Rat markers	Mouse markers	Dog markers
Total T cells	CD45/CD3	CD3	CD3	CD3
Helper T cells	CD45/CD3/CD4	CD3/CD4	CD3/CD4	CD3/CD4
Cytotoxic T cells	CD45/CD3/CD8	CD3/CD8	CD3/CD8	CD3/CD8
B cells	CD45/CD3/CD20	CD3/CD45RA	CD3/CD19	CD3/CD21
NK cells	CD45/CD3/CD16	CD3/CD161	CD3/NK1.1	-
Memory T cells	CD45/CD3/CD4/CD8/CD28/CD95	-	-	-
Regulatory T cells	CD45/CD4/CD8/CD25/FoxP3	CD45/CD4/CD8/CD25/FoxP3	-	-
Dendritic cells	CD66abce/CD16/CD20/HLADR/CD14/CD11c/CD123	-	-	-
γ/δ T cells	CD45/CD3/δγTCR	CD3/δγTCR	-	-
α/β T cells	CD45/CD3/βTCR	CD3/βTCR	-	-
MZB cells	-	CD90/MZB/IgM	-	-
Follicular B cells	-	CD90/MZB/IgM	-	-
Th17	-	CD3/IL-17A	-	-

3 RESULTS

Strain	Matrix	Cell populations	Relative % (Mean±SD)
Beagle	Blood	Total T	62.15 ± 6.32
		T helper	37.70 ± 4.29
		T cytotoxic	18.41 ± 3.68
		B cells	18.78 ± 4.80
	Thymus	CD4+/CD8-	5.73 ± 1.01
		CD4-/CD8+	5.3 ± 1.12
		CD4+/CD8+	37.3 ± 10.34
		CD4-/CD8-	10.1 ± 5.10

Table 1: Dog lymphocyte distribution in whole blood and thymus

Lymphocyte populations in beagle whole blood and thymus(males and females combine) aged between 11 to 13 months.

3 RESULTS (Cont)

Figure 1: Cynomolgus macaque lymphocyte distribution in whole blood

Lymphocyte populations in NHP whole blood (males and females combine) aged between 2.5 to 6 years. Some differences are observed in the lymphocyte populations distribution between Rhesus NHP, Mauritian NHP and Chinese NHP (NM: Naïve memory; CM: central memory; EM: effector memory; T reg: regulatory T cells; mDC: myeloid dendritic cells; pDC: plasmacytoid dendritic cells)

Figure 2: Cynomolgus macaque lymphocyte distribution in lymphoid organs

Lymphocyte populations in NHP lymphoid organs (males and females combine) aged between 2.5 to 6 years. The proportion of the lymphocyte subsets of interest is different in lymphoid organs than in whole blood (NM: Naïve memory; CM: central memory; EM: effector memory)

Figure 3: Rats lymphocyte distribution in whole blood and organs

Lymphocyte populations in rats whole blood and lymphoid organs (males and females combine) aged between 8 to 12 weeks. The lymphocyte distribution in Sprague Dawley, Wistar and Fisher rats are overall similar. As expected, the distribution in whole blood and lymphoid organs is different

3 RESULTS (Cont)

Strain	Cell populations	% (Mean±SD) Blood	% (Mean±SD) Spleen
CD1	Total T	40,86 ± 11,91	33,48 ± 4,01
	T helper	29,73 ± 9,15	24,31 ± 4,67
	T cytotoxic	10,37 ± 3.36	8,41 ± 1,61
	B cells	53,51 ± 12,9	58,09 ± 4,33
	NK cells	4,94 ± 2,69	4,60 ± 2,34
VG	Total T	39,99 ±7,48	34,92 ± 4,36
	T helper	26,26 ± 5,75	20,88 ± 2,96
	T cytotoxic	12,65 ± 2,41	11,84 ± 1,95
	B cells	52,85 ± 8,73	57,92 ± 4,63
	NK cells	5,56 ± 2,54	3,09 ± 0,66

Table 1: Mouse lymphocyte distribution in whole blood and spleen

Lymphocyte populations in mouse whole blood and spleen (males and females combine) aged between 8 to 10 weeks (VG: velocigene mouse with a C57BL6 background)