Selective loss of vaccine-specific memory B cells in a rhesus macaque model of chemotherapy: influence of doxorubicin on immunological memory

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Supplementary data

Table 1.

Treated Animals		Day 0	Nadir* dose 1	Nadir* dose 2	Nadir* dose 3	Day 73	Day 86	Day 101	Day 121	Day 136	Day 175	Day 211
Dx1	WBCs	11.49	3.44	2.72	2.14	13.94	5.94	5.49	4.85	5.64	5.17	4.02
	Neutrophils	10.1	0.3	0.1	0.01	5.6	2.1	1.4	1.4	1.6	0.69	0.9
	Lymphocytes	1.0	1.6	1.6	0.9	0.9	3.0	2.8	3.1	3.7	3.8	2.76
	B cells	0.10	0.13	0.03	-	0.09	-	0.06	0.10	0.15	0.24	0.17
	CD27+ B cells	0.03	0.07	0.01	-	ND	-	0.02	0.01	0.04	0.06	0.05
	Weight	11.2	10.1	9.2	8.8	8.9	9.0	9.1	9.3	9.5	10.5	11.1
Dx2	WBC	5	3.95	2.55	0.62	30.03	8.01	4.28	3.78	4.01	5.8	6.49
	Neutrophils	2.6	0.5	0.5	0.1	21.2	3.0	1.7	1.5	1.2	1.68	2.4
	Lymphocytes	1.9	1.4	0.8	0.5	4.6	3.7	1.9	1.9	2.4	3.49	3.41
	B cells	0.55	0.16	0.04	-	0.08	-	0.08	0.51	0.75	1.36	1.20
	CD27+ B cells	0.32	0.09	0.02	-	0.02	-	0.03	0.04	0.16	0.45	0.44
	Weight	12.2	11.2	9.6	8.5	8.2	8.3	8.4	8.4	8.5	9.2	9.4
Dx3	WBC	7.55	3.59	2.06	1.62	1.62	4.81	4.05	3.88	3.81	4.47	4.12
	Neutrophils	2.1	2.6	0.9	0.1	0.1	3.4	2.7	2.5	2.5	3.03	2.65
	Lymphocytes	4.6	0.7	0.7	0.6	0.7	1.1	1.0	1.1	1.0	1.14	1.11
	B cells	0.46	0.04	0.03	-	0.07	-	0.03	0.05	0.05	0.07	0.07
	CD27+ B cells	0.13	0.01	0.01	-	0.01	-	0.01	0.01	0.01	0.02	0.02
	Weight	10.1	10.1	10.0	9.8	9.6	10.1	10.1	10.4	10.7	11.05	11.4
Dx4	WBC	9.18	2.29	4.39	0.93	3.14	8.53	5.22	6.02	4.86	3.75	4.08
	Neutrophils	7.5	0.9	1.6	0.1	0.9	4.9	3.4	4.1	2.7	2.06	2.62
	Lymphocytes	1.3	1.1	0.8	0.7	1.4	2.8	1.4	1.6	1.9	1.47	1.27
	B cells	0.29	0.10	0.03	-	0.04	-	0.07	0.17	0.29	0.39	0.46
	CD27+ B cells	0.16	0.05	0.01	-	0.004	-	0.05	0.05	0.10	0.13	0.18
	Weight	11.7	11.1	10.7	10.4	10.3	10.7	10.6	10.5	10.7	10.6	10.5
Dx5	WBC	5.04	4.19	3.01	1.78	4.66	9.15	4.11	4.53	6.04	3.43	3.43
	Neutrophils	2.4	1.4	0.2	0.2	0.3	4.8	1.6	1.7	3.7	1.42	1.9
	Lymphocytes	2.1	1.2	1.5	1.4	3.1	2.9	1.6	1.8	1.8	1.53	0.99
	B cells	0.10	0.04	0.02	-	0.03	-	0.02	0.03	0.03	0.04	0.04
	CD27+ B cells	0.06	0.03	0.01	-	0.002	-	0.01	0.01	0.02	0.02	0.02
	Weight	12.5	12.0	11.5	11.0	10.7	10.7	10.5	10.8	10.2	10.3	10.5

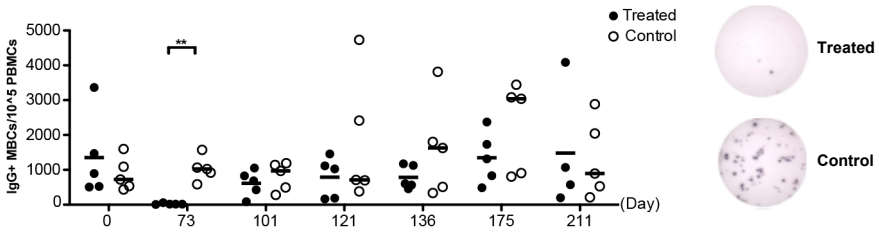
Supplementary data

Control Animals		Day 0	Nadir matched[#] dose 1	Nadir matched[#] dose 2	Nadir matched[#] dose 3	Day 73	Day 86	Day 101	Day 121	Day 136	Day 175	Day 211
Ctrl1	WBCs	9.03	6.74	7.07	5.65	5.15	6.41	7.95	7.79	5.81	6.84	10.15
	Neutrophils	6.2	4.0	5.0	3.4	3.1	4.3	4.9	4.5	3.0	4.1	8.08
	Lymphocytes	2.2	2.3	1.7	1.8	1.8	1.5	2.6	2.6	2.2	2.0	1.56
	B cells	0.57	0.74	0.43	-	0.49	-	0.26	0.28	0.24	0.34	0.19
	CD27+ B cells	0.10	0.19	0.13	-	0.13	-	0.06	0.02	-	0.05	0.05
	Weight	9.7	9.0	8.1	7.9	7.7	7.8	7.9	8.0	7.9	8.4	8.4
Ctrl2	WBC	9.47	8.44	6.97	7.33	9.58	7.31	10.71	6.62	5.96	6.51	6.99
	Neutrophils	6.4	5.1	3.7	3.7	5.3	3.6	5.9	3.8	3.4	3.0	4.03
	Lymphocytes	2.3	3.2	2.4	2.8	3.4	3.0	3.4	2.3	2.1	2.9	2.28
	B cells	0.68	-	0.70	-	0.92	-	0.87	0.60	0.54	0.77	0.61
	CD27+ B cells	0.47	-	0.50	-	0.66	-	0.57	0.27	0.38	0.59	0.47
	Weight	16.3	15.6	15.5	14.8	14.8	14.5	14.7	14.6	14.1	14.0	13.8
Ctrl3	WBC	5.71	7.1	3.44	4.32	6.18	4.27	5.68	3.64	3.97	4.67	4.39
	Neutrophils	2.4	4.9	2.0	2.9	3.6	2.4	3.4	1.7	2.3	2.1	2.76
	Lymphocytes	2.8	2.2	1.3	1.2	2.3	1.5	1.9	1.7	1.5	2.3	1.38
	B cells	0.35	0.27	0.13	-	0.27	-	0.22	0.19	0.16	0.28	0.15
	CD27+ B cells	0.19	0.15	0.07	-	0.17	-	0.13	0.09	0.10	0.18	0.10
	Weight	11.5	10.7	10.4	10.7	10.5	10.6	10.6	10.8	10.3	10.9	10.3
Ctrl4	WBC	9.59	6.61	9.02	13.4	11.61	10.39	14.21	15.74	10.61	8.93	8.22
	Neutrophils	6.8	4.5	4.9	7.5	7.2	4.3	7.2	8.0	4.1	2.8	3.97
	Lymphocytes	1.9	2.0	2.8	3.0	2.2	3.6	3.7	5.1	4.1	4.2	2.74
	B cells	0.51	-	0.16	-	0.26	-	0.36	0.43	0.49	0.72	0.59
	CD27+ B cells	0.35	-	0.11	-	0.17	-	0.30	0.30	0.42	0.63	0.51
	Weight	11.8	11.0	10.6	10.7	10.4	10.3	10.3	9.2	9.3	9.4	9.0
Ctrl5	WBC	6.36	4.22	3.08	4.15	5.03	2.98	4.59	4.16	2.77	4.61	4.57
	Neutrophils	4.8	3.3	2.1	2.5	3.2	1.7	3.1	2.1	1.5	2.3	3.34
	Lymphocytes	1.4	0.9	0.9	1.4	1.5	1.1	1.1	1.6	1.0	1.9	1.08
	B cells	0.12	-	0.08	-	0.12	-	0.10	0.12	0.09	0.10	0.08
	CD27+ B cells	0.06	-	0.03	-	0.06	-	0.04	0.02	0.04	0.05	0.04
	Weight	9.9	9.3	9.7	9.9	9.7	9.8	10.1	10.5	10.2	10.7	10.6

Supplementary table 1. Peripheral blood cell counts in Doxorubicin (Dx, n=5) and saline treated control (Ctrl, n=5) animals. Absolute counts ($x10^9$/l) for white blood cells (WBC), neutrophils and lymphocytes were measured in fresh EDTA-anticoagulated blood with COBAS INTEGRA-400+ (Roche). Simultaneously, absolute B cell counts were calculated by multiplying %CD20+ lymphocytes determined by flow cytometric analysis with absolute lymphocyte counts. Similarly, memory B-cell counts were determined by multiplying %CD27+ B cells with absolute B cell counts. *Nadir occurred between 10-15 days after each Dx dose. [#]Nadir –matched values in control animals origin from day 14 after each saline dose. Weight=kg. ND=not detectable,-=data missing

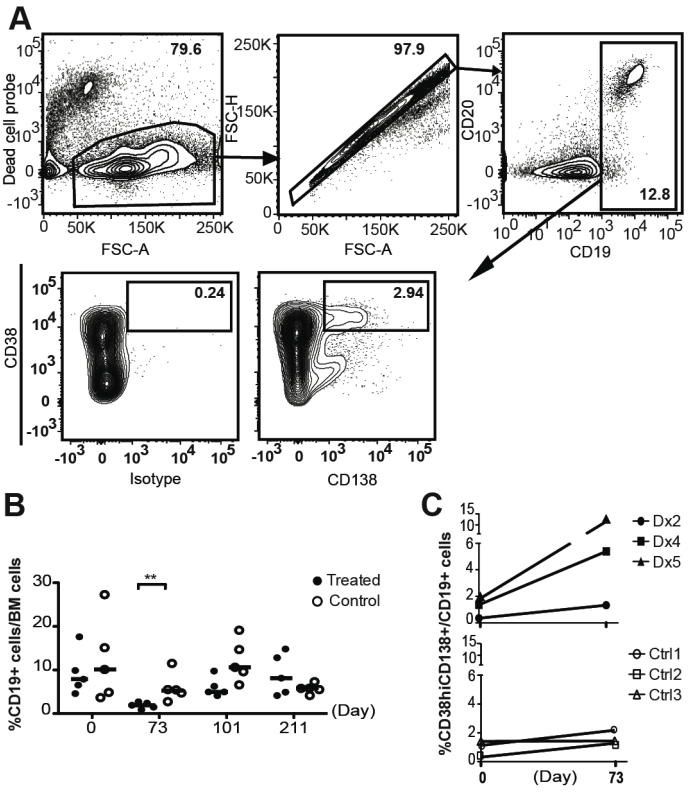
Supplementary data

Figure 1.



Supplementary figure 1. Proportion of total IgG+ memory B cell (MBC) determined by ELISPOT. Values depict ratio of IgG+ cells per 10^5 PBMCs. Representative ELISPOT wells from day 73. Horizontal lines represent median. The frequencies of MBCs were significantly lower in the treated group on day 73 (p=0.008).

Figure 2.



Supplementary figure 2. Flow cytometric analysis of the BM compartment following Doxorubicin treatment. (A) Representative gating strategy where BM B cells were defined as live $CD19^{+}$, $CD20^{+/-}$ single cells and BMPCs were defined as $CD38^{hi}$, $CD138^{+}$ B cells. (B) Proportions of CD19+ BM cells among live single BM cells. **p<0.01. (C) Individual variations in BMPC proportions between day 0 and 73 in chemotherapy (Dx) and saline (Ctrl) treated animals (n=3/group).