Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Mass Cytometric Study on the Heterogeneity in Cellular Association and Cytotoxicity of Silver Nanoparticles in Primary Human Immune Cells

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Supplementary Methods

Hydrodynamic size and zeta potential characterization

Dispersions of AgNPs were prepared in DI water and RPMI complete media, which was RPMI-1640 medium (Lonza™ BioWhittaker™, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). The hydrodynamic sizes and zeta potentials were measured using a Malvern Zetasizer (Nano-ZS, Malvern Instruments Ltd, UK) following manufacturer' instructions. Measurements were performed in triplicate.

Biodistribution analysis

The biodistribution of AgNPs in PBMCs was analysed by TEM. PBMCs were isolated from whole blood as described in the "Experimental" section. Cells were then incubated with $^{PVP}Ag^{10}$ and $^{PVP}Ag^{20}$ NPs at 10 μg/mL for 3 h at 37°C and 5% CO_2. To prepare thin-sectioned specimens for TEM analysis, cells were fixed with 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M PBS, pH 7 for 3 h at 4°C and then washed with PBS. After that, the samples were post-fixed in 1% osmium tetroxide (Sigma Aldrich, USA) for 2 h at room temperature and washed again with PBS. Next, dehydration was performed by a graded ethanol series (50%, 60%, 70%, 80%, 90% and 100% ethanol) for 1 h each time. Cells were subsequently infiltrated by mixtures of ethanol and propylene oxide at 2:1, 1:1, 1:2 and 0:1 ratios for 1 h each, and then by mixtures of propylene oxide and EPON epoxy resin (Structure Probe, USA) at 2:1, 1:1 and 1:2 ratios for 1 h each. Then, cells were embedded in epoxy resin and loaded into capsules to polymerize at 60°C for 48 h. Thin-sectioning was performed using a Leica EM UC7 ultramicrotome (Leica Microsystems, Germany) and collected on copper grids. Images were acquired using a JEM-1400 Flash transmission electron microscope (JEOL Ltd., Japan) at 120 kV.

Supplementary Figures

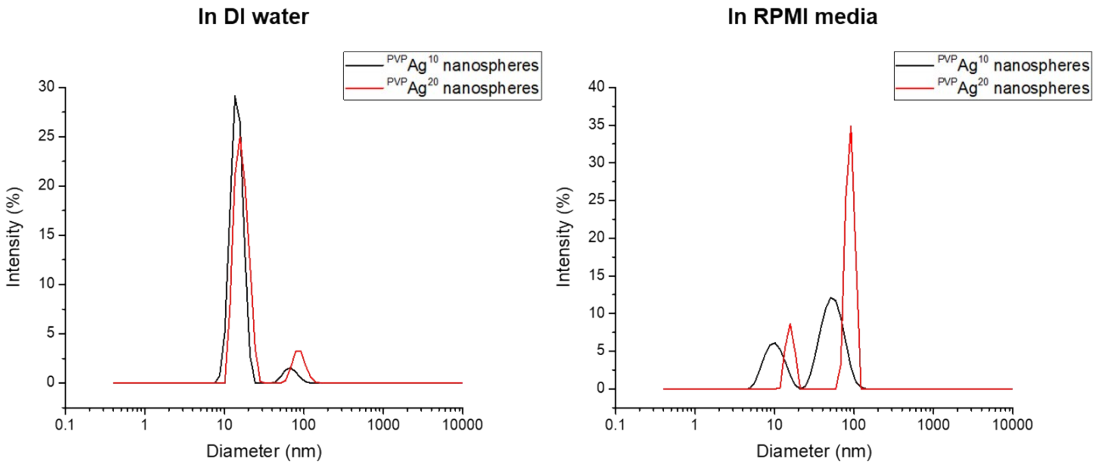

Figure S1. Hydrodynamic size distributions of the AgNPs in DI water and RPMI media. Distribution profiles were averaged from triplicate measurements.

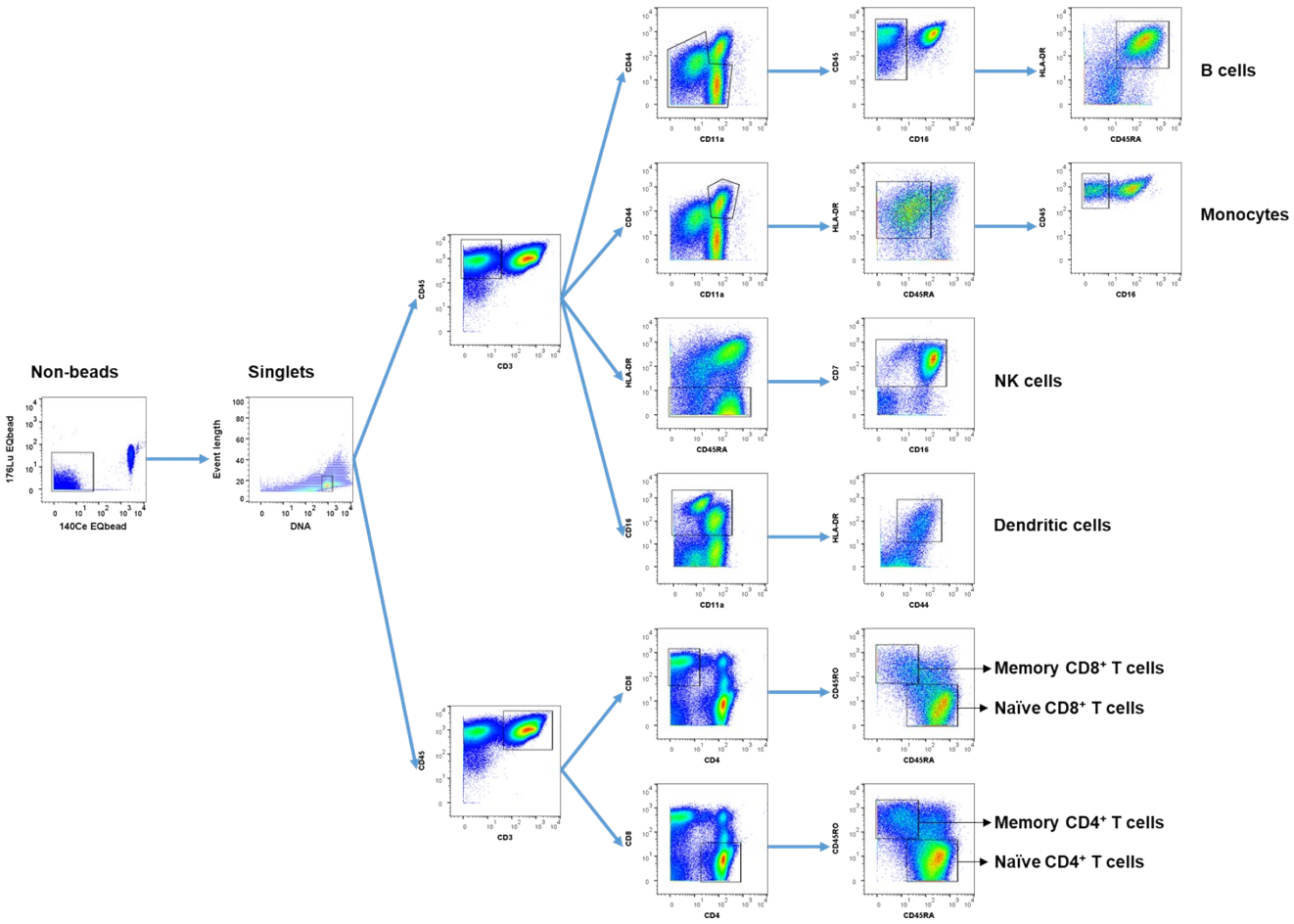

Figure S2. Gating strategies of the major immune populations in peripheral blood mononuclear cells.

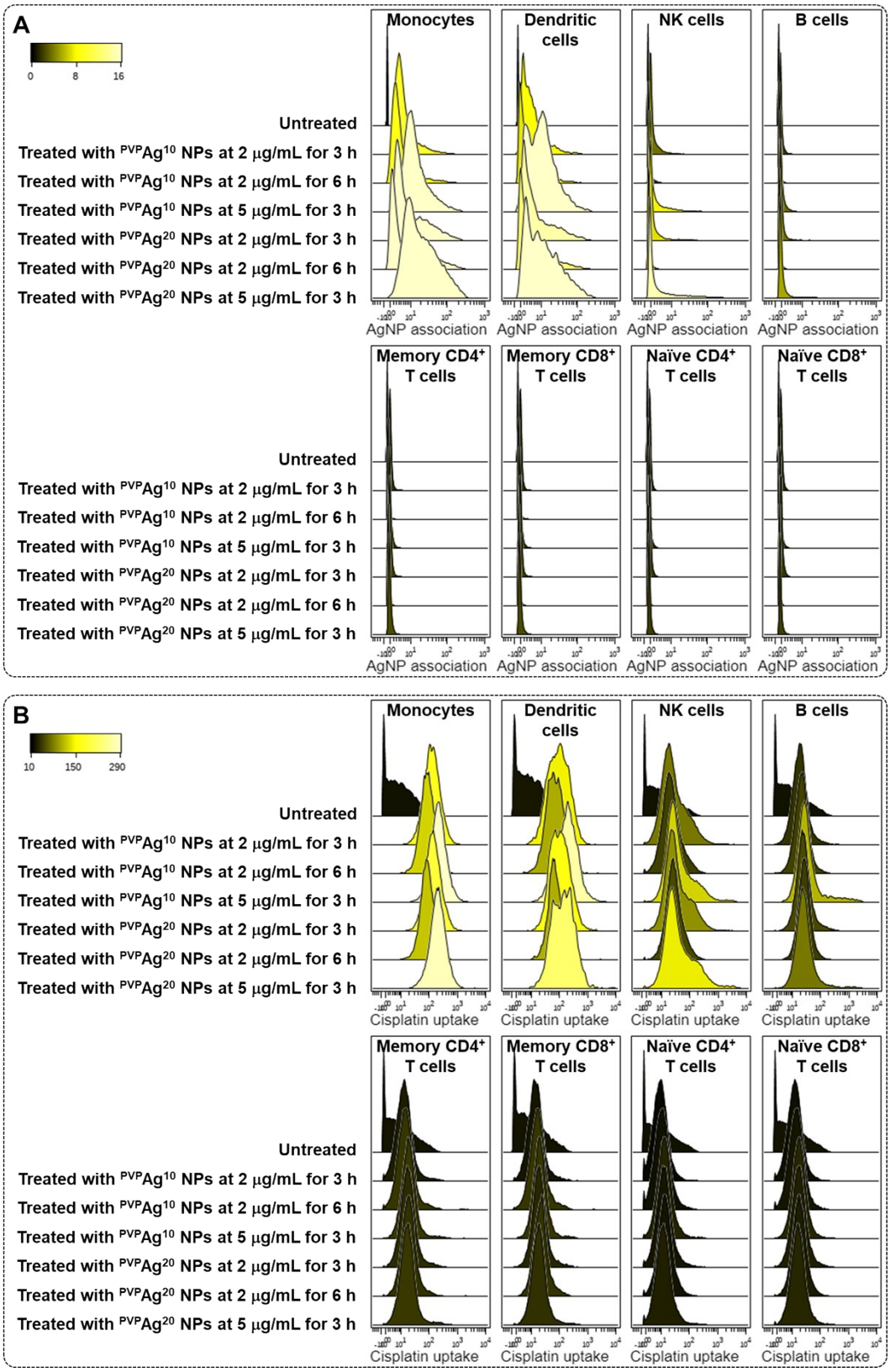

Figure S3. Histograms of the (A) cellular AgNP association (in femtogram) and (B) cisplatin uptake of each cell type in each exposure condition. The colour scale represents the mean intensity of silver in (A) and cisplatin in (B).

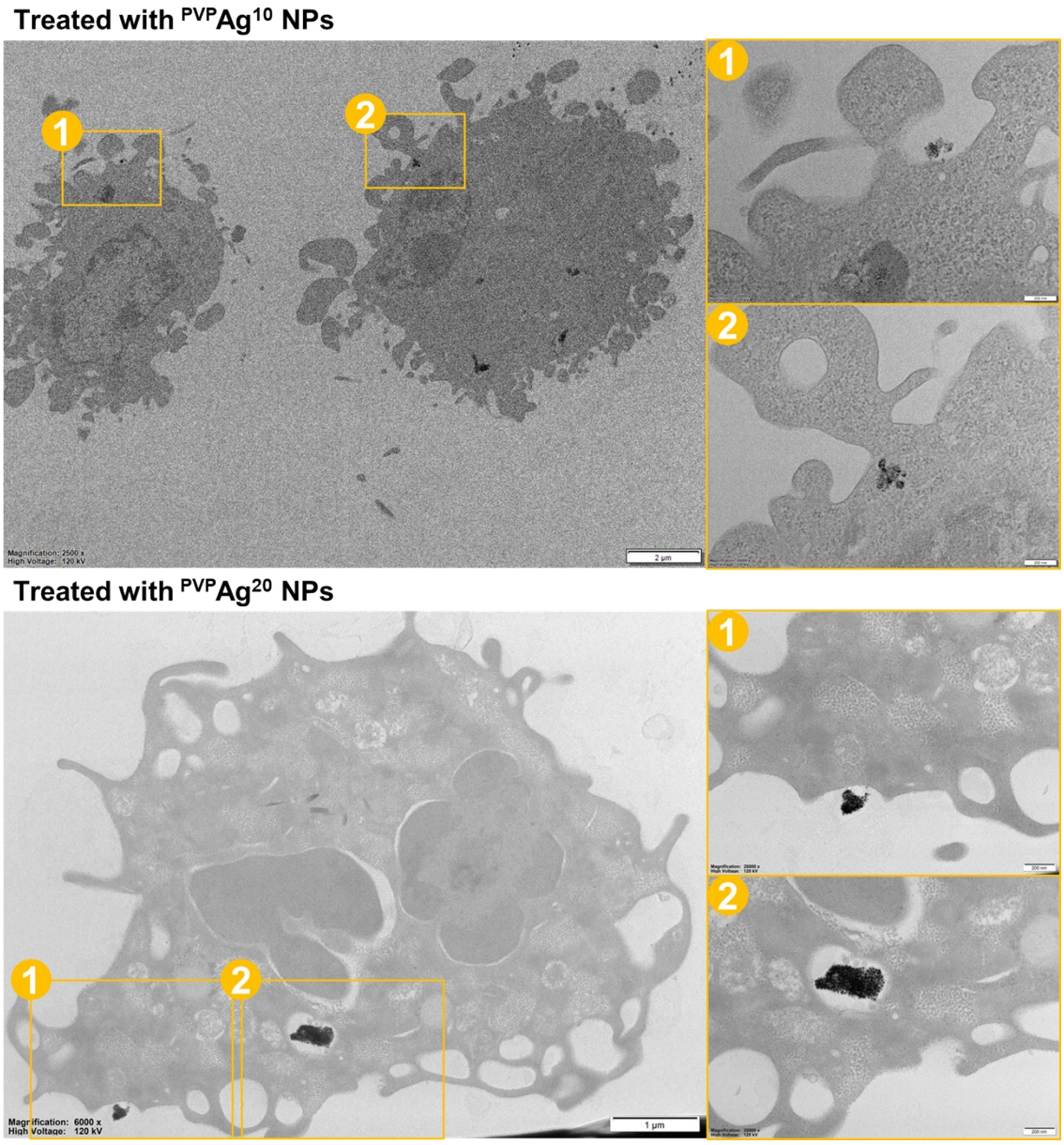

Figure S4. Thin-section TEM images of the biodistribution of AgNPs in PBMCs. Scale bars of the large panels represent 2 µm (upper image) or 1 µm (lower image) and scale bars of small panels represent 200 nm. AgNPs could be found in agglomerates that were either bound on the membrane, internalised in the cytosol or in endosomes. However, based on TEM images, the various cell types of PBMCs could not be distinguished.

Supplementary Tables

Table S1. Core size, hydrodynamic size and zeta potential of the AgNPs. Nominal core sizes were referenced from manufacturer's specifications. Hydrodynamic size and zeta potential were measured by Malvern Zetasizer (Nano-ZS, Malvern Instruments Ltd, UK). Description of these measurements is presented in Supplementary Methods.

Nanoparticles	Diameter (nm)		Zeta potential (mV)	
	Core size (in DI water)[a]	Hydrodynamic size (in RPMI media)[b]	In DI water[b]	In RPMI media[b]
$^{PVP}Ag^{10}$ nanospheres	10 ± 2	41 ± 13	-31.4 ± 1.1	-8.3 ± 0.1
$^{PVP}Ag^{20}$ nanospheres	21 ± 4	75 ± 9	-27.4 ± 1.6	-8.6 ± 1.7

[a] Confidence interval indicates the standard deviation of the size distribution.

[b] Confidence interval indicates the standard deviation of triplicate measurements.

Table S2. Ratios of cisplatin uptake to cellular AgNP association.

Cell type	Replication 1	Replication 2	Replication 2	Mean ± SEM
Treated with $^{PVP}Ag^{10}$ NPs at 2 μg/mL for 3 h				
Monocytes	15.50	15.60	15.63	15.58 ± 0.07
Dendritic cells	15.18	14.41	14.61	14.73 ± 0.40
NK cells	16.02	15.97	13.90	15.30 ± 1.21
B cells	10.65	11.15	11.15	10.98 ± 0.29
Memory $CD4^+$ T cells	3.54	3.53	5.27	4.11 ± 1.00
Memory $CD8^+$ T cells	1.28	1.27	4.00	2.18 ± 1.58
Naïve $CD4^+$ T cells	4.66	4.61	1.25	3.51 ± 1.95
Naïve $CD8^+$ T cells	1.58	1.57	1.12	1.42 ± 0.27
Treated with $^{PVP}Ag^{10}$ NPs at 2 μg/mL for 6 h				
Monocytes	11.99	11.92	11.88	11.93 ± 0.06
Dendritic cells	11.81	10.87	11.21	11.30 ± 0.47
NK cells	12.58	12.53	10.60	11.90 ± 1.13
B cells	22.48	22.60	15.07	20.05 ± 4.31
Memory $CD4^+$ T cells	27.42	27.35	17.93	24.23 ± 5.46
Memory $CD8^+$ T cells	27.74	27.75	20.03	25.18 ± 4.46
Naïve $CD4^+$ T cells	2.11	2.15	6.08	3.44 ± 2.28
Naïve $CD8^+$ T cells	2.73	2.71	5.73	3.72 ± 1.73
Treated with $^{PVP}Ag^{10}$ NPs at 5 μg/mL for 3 h				

Monocytes	13.30	13.45	13.37	13.38 ± 0.08
Dendritic cells	15.16	14.85	14.92	14.98 ± 0.16
NK cells	13.95	13.95	12.90	13.60 ± 0.61
B cells	26.47	26.44	17.70	23.54 ± 5.06
Memory $CD4^+$ T cells	10.92	11.09	10.22	10.74 ± 0.46
Memory $CD8^+$ T cells	11.34	11.38	10.82	11.18 ± 0.31
Naïve $CD4^+$ T cells	6.75	6.70	9.54	7.66 ± 1.62
Naïve $CD8^+$ T cells	8.94	8.94	9.09	8.99 ± 0.09
Treated with $^{PVP}Ag^{20}$ NPs at 2 μg/mL for 3 h				
Monocytes	9.03	9.12	9.13	9.09 ± 0.06
Dendritic cells	9.63	9.17	9.10	9.30 ± 0.29
NK cells	9.89	9.86	8.62	9.46 ± 0.72
B cells	8.72	8.79	6.40	7.97 ± 1.36
Memory $CD4^+$ T cells	7.44	7.42	7.24	7.37 ± 0.11
Memory $CD8^+$ T cells	6.28	6.27	6.53	6.36 ± 0.14
Naïve $CD4^+$ T cells	1.07	1.08	3.98	2.05 ± 1.67
Naïve $CD8^+$ T cells	4.47	4.47	5.52	4.82 ± 0.61
Treated with $^{PVP}Ag^{20}$ NPs at 2 μg/mL for 6 h				
Monocytes	4.98	5.05	4.90	4.98 ± 0.08
Dendritic cells	6.88	6.48	6.75	6.70 ± 0.20
NK cells	6.45	6.43	5.57	6.15 ± 0.50
B cells	11.32	11.49	5.92	9.58 ± 3.17
Memory $CD4^+$ T cells	17.41	17.30	8.57	14.43 ± 5.07
Memory $CD8^+$ T cells	17.77	17.46	9.96	15.06 ± 4.42
Naïve $CD4^+$ T cells	0.88	0.95	3.62	1.82 ± 1.57
Naïve $CD8^+$ T cells	6.13	6.10	4.93	5.72 ± 0.68
Treated with $^{PVP}Ag^{20}$ NPs at 5 μg/mL for 3 h				
Monocytes	7.57	7.64	7.66	7.62 ± 0.05
Dendritic cells	9.65	9.42	9.38	9.48 ± 0.15
NK cells	7.96	7.97	7.42	7.78 ± 0.31
B cells	10.78	10.85	6.49	9.38 ± 2.50
Memory $CD4^+$ T cells	8.76	8.77	7.67	8.40 ± 0.63
Memory $CD8^+$ T cells	6.74	6.76	5.84	6.45 ± 0.52
Naïve $CD4^+$ T cells	3.73	3.77	5.39	4.30 ± 0.95
Naïve $CD8^+$ T cells	5.20	5.20	5.25	5.22 ± 0.03