



Article

Interaction between Macrophages and Nanoparticles: In Vitro 3D Cultures for the Realistic Assessment of Inflammatory Activation and Modulation of Innate Memory

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Table S1. Primary cytokine production by monocytes in 2D vs. 3D cultures.

Culture conditions	Cytokine	Cytokine production ¹					
		Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
2D							
medium	TNF α	nt ²	0.06 (0.10)	0.21 (0.02)	0	0	0
LPS		nt	0.82 (0.28)	1.95 (0.53)	3.15 (0.31)	3.15 (0.11)	15.17 (0.76)
AuNP		nt	0.02 (0.01)	0.26 (0.03)	0.12 (0.02)	0.09 (0.09)	0
medium	IL-6	0.24 (0.02)	0.15 (0.04)	0.81 (0.09)	0	0	0
LPS		2.19 (0.19)	1.78 (0.31)	3.99 (0.23)	2.75 (0.12)	6.20 (2.41)	6.11 (0.19)
AuNP		0.25 (0.07)	0.12 (0.03)	0.85 (0.11)	0	0.23 (0.25)	0
medium	IL-8	2.43 (0.08)	8.15 (1.70)	3.65 (0.02)	0.58 (0.14)	0.08 (0.11)	0.60 (0.02)
LPS		10.11 (7.50)	22.38 (3.40)	11.55 (0.80)	13.88 (1.77)	18.75 (4.84)	81.85 (9.56)
AuNP		2.41 (0.01)	8.46 (2.40)	3.85 (0.07)	0.13 (0.03)	7.97 (6.72)	1.01 (0.42)
medium	IL-1Ra	5.12 (0.85)	3.04 (0.44)	4.77 (0.95)	1.13 (0.11)	0.37 (0.08)	1.66 (0.12)
LPS		4.59 (0.32)	3.88 (1.05)	6.20 (1.04)	0.98 (0.01)	2.60 (0.17)	3.05 (0.21)
AuNP		4.12 (1.13)	3.35 (0.59)	5.43 (0.40)	0.94 (0.03)	0.79 (0.17)	1.85 (0.28)
3D							
medium	TNF α	0.15 (0.02)	0.05 (0.01)	0.30 (0.01)	0	0	0
LPS		0.93 (0.13)	1.21 (0.18)	1.11 (0.18)	3.02 (0.13)	3.06 (0.54)	9.50 (0.44)
AuNP		0	0.04 (0.01)	0.25 (0.04)	0.07 (0.01)	0.09 (0.08)	0
medium	IL-6	0.47 (0.04)	0.19 (0.06)	1.66 (0.06)	0.05 (0.01)	0	0.06 (0.04)
LPS		3.57 (0.11)	2.58 (0.67)	3.56 (0.22)	2.55 (0.02)	3.93 (1.68)	4.45 (0.60)
AuNP		0.42 (0.19)	0.21 (0.08)	1.00 (0.09)	0.30 (0.03)	0.15 (0.15)	0.15 (0.09)
medium	IL-8	5.14 (0.10)	nt	5.61 (0.18)	nt	5.35 (1.58)	3.89 (1.50)
LPS		17.71 (1.70)	nt	15.26 (0.27)	nt	32.88 (11.08)	121.55 (21.1)
AuNP		4.74 (0.04)	nt	5.85 (0.07)	nt	12.00 (2.37)	9.60 (2.20)
medium	IL-1Ra	3.39 (0.53)	4.99 (1.06)	2.94 (0.99)	0.52 (0.02)	1.60 (0.34)	3.14 (1.14)
LPS		4.17 (0.60)	5.47 (0.28)	3.90 (0.23)	5.22 (1.20)	2.43 (0.66)	3.70 (1.04)
AuNP		6.62 (1.66)	3.75 (1.11)	4.68 (2.39)	2.53 (0.20)	2.22 (0.44)	4.44 (1.14)

¹ Cytokines were measured in the supernatant of 2D and 3D monocyte cultures exposed to culture medium alone for 24 h. Results are the mean values of 2-8 replicate determinations and are expressed in ng/10⁶ cells. SD is shown in parentheses. Undetectable cytokine levels are shown as zero.

² nt = not tested

Table S2. Baseline cytokine production by unprimed and primed monocytes after 6-day resting in 2D vs. 3D cultures.

Cytokine	Cytokine production ¹																	
	Unprimed						LPS-primed						AuNP-primed					
	D1	D2	D3	D4	D5	D6	D1	D2	D3	D4	D5	D6	D1	D2	D3	D4	D5	D6
2D																		
TNF α	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IL-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IL-8	0	0	0	0	0	0	0.14 (0.08)	0	0	0	0.17 (0.07)	0	0	0	0	0	0.08 (0.02)	0
IL-1Ra	0.99 (0.72)	0.70 (0.17)	0.49 (0.15)	0.29 (0.01)	0.27 (0.11)	1.07 (0.19)	0.36 (0.10)	0.32 (0.16)	0.37 (0.44)	0.29 (0.03)	0.37 (0.48)	0.09 (0.02)	0.46 (0.11)	0.63 (0.10)	1.18 (0.86)	0.47 (0.03)	0.17 (0.06)	1.41 (0.63)
3D																		
TNF α	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IL-6	0	0	0	0	0	0	0	0	0.07 (0.01)	0	0	0	0	0	0	0	0	0
IL-8	2.91 (0.35)	0.65 (0.18)	2.08 (0.05)	0.58 (0.07)	0	0	3.48 (0.15)	2.46 (0.27)	3.09 (0.28)	2.70 (0.80)	0.29 (0.04)	2.50 (1.59)	1.38 (0.08)	1.87 (0.39)	2.07 (0.10)	0.76 (0.30)	0	0
IL-1Ra	4.17 (0.39)	0.74 (0.44)	0.67 (0.18)	2.67 (2.00)	0.77 (0.32)	2.33 (0.82)	1.59 (0.30)	1.33 (0.04)	1.37 (1.10)	4.19 (1.46)	1.32 (0.33)	5.74 (3.38)	0.97 (0.46)	1.85 (1.30)	1.50 (0.23)	3.72 (1.82)	0.53 (0.19)	4.86 (0.12)

¹ Cytokines were measured in the supernatant of 2D and 3D monocyte cultures that were previously primed for 24 h with culture medium alone (unprimed) or containing LPS (1 ng/mL) or AuNP (1 μ g/mL), cultured with culture medium alone for 6 additional days, and eventually exposed for further 24 h to fresh culture medium. Results are the mean values of 2-4 replicate determinations and are expressed in ng/10⁶ cells. SD is shown in parentheses. Undetectable cytokine levels are shown as zero.

Table S3. Secondary cytokine production by unprimed and primed monocytes challenged with LPS after 6-day resting in 2D vs. 3D cultures.

Cytokine	Cytokine production ¹																	
	Unprimed						LPS-primed						AuNP-primed					
	D1	D2	D3	D4	D5	D6	D1	D2	D3	D4	D5	D6	D1	D2	D3	D4	D5	D6
2D																		
TNF α	nt ²	0.08 (0.02)	0.33 (0.02)	0.26 (0.05)	0.19 (0.07)	4.45 (0.19)	nt	0	0.07 (0.02)	0	0.02 (0.01)	0	nt	0.26 (0.06)	0.39 (0.09)	0.26 (0.16)	0.23 (0.03)	3.80 (0.32)
IL-6	0.03 (0.01)	0.46 (0.11)	0.17 (0.03)	0.16 (0.01)	0.18 (0.07)	2.72 (0.09)	0	0.08 (0.01)	0.03 (0.01)	0	0.03 (0.01)	0.12 (0.08)	0.02 (0.01)	0.54 (0.07)	0.21 (0.04)	0.14 (0.11)	0.18 (0.04)	2.46 (0.08)
IL-8	8.15 (1.10)	13.49 (2.72)	4.38 (0.26)	5.67 (1.19)	6.86 (4.97)	3.10 (0.79)	1.23 (0.47)	7.71 (2.08)	1.35 (0.13)	4.64 (1.74)	3.72 (1.45)	6.30 (1.67)	6.42 (0.69)	15.99 (2.15)	5.22 (1.43)	4.25 (0.41)	6.64 (0.83)	5.15 (3.03)
IL-1Ra	2.10 (1.05)	0.88 (0.20)	0.61 (0.23)	0.52 (0.10)	0.58 (0.16)	3.04 (0.46)	0.66 (0.09)	0.20 (0.03)	0.88 (0.11)	0.27 (0.11)	0.23 (0.38)	0.15 (0.06)	1.05 (0.14)	0.87 (0.10)	1.52 (0.68)	0.43 (0.20)	0.62 (0.2s2)	3.35 (0.92)
3D																		
TNF α	nt	0.45* (0.22)	1.01* (0.06)	2.71* (0.28)	1.09* (0.32)	3.22* (0.30)	nt	0.16* (0.12)	0.24* (0.02)	0.68* (0.09)	0.14* (0.02)	1.38* (0.12)	nt	0.66* (0.15)	0.33 (0.08)	1.83* (0.60)	0.68* (0.06)	4.91 (1.54)
IL-6	0.08 (0.05)	0.26 (0.16)	0.58* (0.04)	0.62* (0.07)	1.25* (0.52)	1.43* (0.03)	0.39 (0.32)	0.24 (0.15)	0.57* (0.24)	0.24* (0.10)	0.46* (0.05)	0.42* (0.04)	0.30* (0.12)	0.43 (0.11)	0.24 (0.07)	0.33 (0.14)	0.87* (0.06)	1.84 (0.63)
IL-8	18.41* (5.40)	15.81 (7.33)	12.77* (2.14)	18.44* (1.09)	16.24* (3.88)	2.11 (1.27)	26.03* (6.65)	20.34* (5.80)	10.77* (0.86)	16.90* (0.95)	13.16* (1.92)	19.60* (8.83)	20.70* (2.82)	22.34* (2.34)	9.29* (0.45)	15.47* (0.53)	11.34* (0.45)	7.88 (3.70)
IL-1Ra	4.86* (3.01)	2.42* (0.97)	3.23* (0.60)	5.30* (0.32)	1.42* (0.38)	1.59* (0.43)	8.32 ^v (4.73)	2.33* (1.50)	1.82* (0.80)	4.00* (0.65)	4.27* (0.72)	3.54* (0.52)	3.47* (0.37)	3.73* (0.34)	1.69* (0.27)	2.96* (2.19)	1.57* (0.79)	4.47 (3.20)

¹ Cytokines were measured in the supernatant of 2D and 3D monocyte cultures that were previously primed for 24 h with culture medium alone (unprimed), LPS (1 ng/mL) or AuNP (1 μ g/mL), then cultured with fresh medium for 6 additional days, and eventually challenged with LPS (10 ng/mL) for 24 h. Results are the mean values of 2-4 replicate determinations and are expressed in ng/10⁶ cells. SD is shown in parentheses. Undetectable cytokine levels are shown as zero.

nt = not tested.

* $p < 0.05$ between 2D and 3D