

Supplementary material

Figure S1 Induction of MHC expression by IFN- γ desorbed from Alhydrogel[®]

Histograms of THP-1 cells showing the expression of MHC after; (A) Addition of 37.5 ng IFN- γ into the culture medium, giving the concentration of 8.6 ng IFN- γ /ml, (B) Culture in medium without any addition of IFN- γ , (C) Addition of 32 μ g Alhydrogel[®] containing at most 11.7 ng adsorbed IFN- γ , (D) Addition of 200 μ g Alhydrogel[®] containing no adsorbed IFN- γ

The cells were cultured over-night, day 1, removed from the TC inserts and stained with antibodies against MHC class I and class II. New TC insert with cells were added to the previously used medium. The cells were cultured over-night, day 2, removed from the TC inserts and stained with antibodies against MHC class I and class II. (1) Staining with an isotype control antibody, (2) Cells at day 1 stained with an APC labelled antibody against human MHC class I, (3) Cells at day 2 stained with an APC labelled antibody against human MHC class I, (4) Cells at day 1 stained with a FITC labelled antibody against human MHC class II, (5) Cells at day 2 stained with a FITC labelled antibody against human MHC class II.

Black line showing and representing the peak fluorescence channel from cells cultured after the addition of 37.5 ng IFN- γ to the culture medium.

Table S1

Location of controls and cytokines on the array membrane

The spot on the upper left has the coordinate A1 and the spot on the lower right has the coordinate E20

Coordinate	Target/control	Coordinate	Target/control
A1, A2	Reference Spots	C7, C8	IL-4
A3, A4	CCL1/I-309	C9, C10	IL-5
A5, A6	CCL2/MCP-1	C11, C12	IL-6
A7, A8	MIP-1 α /MIP-1 β	C13, C14	IL-8
A9, A10	CCL5/RANTES	C15, C16	IL-10
A11, A12	CD40 Ligand	C17, C18	IL-12 p70
A13, A14	Complement C5/C5a	D3, D4	IL-13
A15, A16	CXCL1/GRO α	D5, D6	IL-16
A17, A18	CXCL10/IP-10	D7, D8	IL-17A
A19, A20	Reference Spots	D9, D10	IL-17E
B3, B4	CXCL11/I-TAC	D11, D12	IL-18/IL-1F4
B5, B6	CXCL12/SDF-1	D13, D14	IL-21
B7, B8	G-CSF	D15, D16	IL-27
B9, B10	GM-CSF	D17, D18	IL-32a
B11, B12	ICAM-1/CD54	E1, E2	Reference Spots
B13, B14	IFN- γ	E3, E4	MIF
B15, B16	IL-1 α /IL-1F1	E5, E6	Serpin E1/PAI-1
B17, B18	IL-1 β /IL-1F2	E7, E8	TNF- α
C3, C4	IL-1ra/IL-1F3	E9, E10	TREM-1
C5, C6	IL-2	E19, E20	Negative Control

Table S2. Culture of THP-1 cells in the presence of added IFN- γ and Alhydrogel® containing adsorbed γ -IFN.

THP-1 cells cultured in tissue culture (TC) inserts and stained with antibodies against human MHC class I (anti HLA-ABC) and against MHC class II (anti HLA-DR, DP, DQ) after co-culture with various concentrations of directly added IFN- γ or Alhydrogel® containing adsorbed γ -IFN. Concentrations of IFN- γ in the culture medium were determined by ELISA.

Expression of MHC presented as the ratio between mean fluorescence intensity (MFI) after staining with labelled antibody against MHC from cells cultured in the presence of IFN- γ or Alhydrogel® and the MFI obtained after staining THP-1 cells cultured in TC insert and medium (R10 control). The Table shows representative results from one of three independent experiments performed

Sample	Day 0		Day 1			Day 2		
	Added IFN- γ (ng) in a total volume of 4ml	Medium conc ^a (pg/ml)	Ratio MFI sample / MFI R10		Medium conc ^b (pg/ml)	Ratio MFI sample / MFI R10		Medium conc (pg/ml)
			MHC-I	MHC-II		MHC-I	MHC-II	
Medium control, R10	0	0	1.0	1.0	0	1.0	1.0	0
Free IFN- γ	7.5	1700	1.3	18.7	335	1.8	12.7	237
Free IFN- γ	37.5	8600	1.4	27.8	1639	1.8	24.6	1894
200 μ g Alhydrogel®	0	0	1.0	1.0	0	1.0	1.0	0
6.7 μ g Alhydrogel®*IFN- γ	2.5 ^c	266	1.3	9.7	103	1.6	5.0	84
32 μ g Alhydrogel®*IFN- γ	11.7 ^c	576	1.4	21.3	542	1.9	18.5	620

^a Concentration determined by ELISA before addition of TC insert and first portion of THP-1 and thus adding an additional 1 ml to the well.

^b Concentration determined by ELISA before addition of a new TC insert and second portion of THP-1 and thus adding an additional 1 ml to the well.

^c Calculated assuming 100% recovery of Alhydrogel® after loading with IFN- γ and washing