

Multiplex cytokine assays. Cytokines were measured using Bio-Plex human cytokine multiplex kits (Bio-Rad, Hercules, CA). Calibration curves from recombinant cytokine standards were prepared with threefold dilution steps in the same matrix as the culture supernatants (RPMI 1640 medium containing 10% FBS). High and low spikes (supernatants from stimulated human dendritic cells) were included to determine cytokine recovery. Standards and spikes were measured in triplicate, samples were measured once, and blank values were subtracted from all readings. All assays were carried out directly in a 96-well filtration plate (Millipore, Billerica, MA) at room temperature and protected from light. Briefly, wells were pre-wet with 100 μ l PBS containing 1% BSA, then beads together with a standard, sample, spikes, or blank were added in a final volume of 100 μ l, and incubated together at room temperature for 30 min with continuous shaking. Beads were washed three times with 100 μ l PBS containing 1% BSA and 0.05% Tween 20. A cocktail of biotinylated antibodies (50 μ l/well) was added to beads for a further 30 min incubation with continuous shaking. Beads were washed three times, then streptavidin-PE was added for 10 min. Beads were again washed three times and resuspended in 125 μ l of PBS containing 1% BSA and 0.05% Tween 20. The fluorescence intensity of the beads was measured in using the Bio-Plex array reader. Bio-Plex Manager software with five-parametric-curve fitting (Bio-Rad technical note 2861 at www.bio-rad.com) was used for data analysis.

For Publications we ask you to consider two possibilities:

1. If you could not have done the studies without our consultation and expertise, then please include the Director (Jacqueline Y. Channon) as an author.
2. Otherwise, please acknowledge the IML as follows:

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