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Maintaining dendritic cell viability in culture

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ABSTRACT

When mouse dendritic cells (DCs) are isolated from tissues, purified and placed in a nutritive culture they die more rapidly than would be expected from their normal turnover *in vivo*. This can distort culture assays of DC function. We therefore tested several approaches to prolonging DC survival in culture. Of several cytokines tested granulocyte-macrophage colony stimulating factor was most effective at preserving the viability of conventional DCs (cDCs) but was ineffective for plasmacytoid DCs (pDCs). Surprisingly, Fms-like tyrosine kinase 3 ligand, crucial for DC development, produced only a marginal improvement in DC survival in culture, and interleukin-3, reported to prevent apoptosis of human pDCs, produced only a minor improvement in survival of mouse DCs. Genetic manipulation of cell death pathways was also tested, to avoid activation effects exerted by cytokine signalling. The isolation of DCs from mice overexpressing Bcl-2 was especially effective in maintaining pDC viability but gave a lesser improvement in cDC viability. DCs isolated from *Bim*$^{-/-}$ *Noxa*$^{-/-}$ mice also showed improved culture survival, but in this case with pDCs showing the least improvement.

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1. Introduction

To determine the specific role of component cells of the immune system, particular cell types are often isolated and purified from lymphoid tissues, then their functional capacity analysed in culture. This reductionist approach can yield valuable information, but removal from the tissue environment may also distort the behaviour of the cells away from their normal *in vivo* functions. This has been a significant problem in the study of dendritic cells (DCs). One culture artefact we have previously studied is the "spontaneous" activation of mouse spleen DCs that occurs when these DCs are purified then placed in culture; we found that this was largely due to the artificially close proximity of the pure DCs to each other, resulting in a form of self activation (Vremec et al., 2011). A more serious problem is the fast rate of death of certain DC subtypes in culture; this can distort *in vitro* assays of such functions as antigen processing and presentation. This problem is especially troublesome with mouse plasmacytoid DCs (pDCs) that survive for weeks *in vivo* (O'Keeffe et al., 2002) but die very rapidly in culture (Sathe et al., 2013). It is also a major problem with the CD8$^+$ subset of conventional DCs (cDCs) isolated from mouse spleen. Although these DCs already have a rapid turnover *in vivo* (Kamath et al., 2000), this is increased *in vitro* to the point that even overnight culture produces a marked loss of cell viability.

We now test several cytokines involved in DC development and function *in vivo* to determine their effectiveness in maintaining DC viability in culture. We also test some mice with genetic modifications of the cell death pathways (Strasser et al., 2011), to determine if such mice could provide a convenient source of DCs with an extended culture survival time. The aim was not to expand DC numbers or promote DC development, but rather to maintain viability long enough to allow tests of DC function in culture.

2. Materials and methods

2.1. Mice

All mice were bred at the Walter and Eliza Hall Institute (WEHI). Most experiments used C57BL/6J Wehi mice, 6–8 weeks old. The *Bim*$^{-/-}$ *Noxa*$^{-/-}$ mice (Fuertes Marraco et al., 2011) were selecting after crossing *Bim*$^{+/-}$ *Noxa*$^{-/-}$ mice with *Bim*$^{+/-}$ *Noxa*$^{-/-}$ or *Bim*$^{+/-}$ *Noxa*$^{+/-}$ mice. The *Vav-Bcl2* transgenic mice (Vaux et al., 1998) were selected after crossing male transgenics with C57BL/6 females. All procedures were approved by the WEHI Animal Ethics Committee.

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2.2. Isolation of spleen DCs

Spleens were pooled, chopped, digested with collagenase-DNase, light density cells isolated by a density cut, non-DCs depleted using immunomagnetic beads, then the individual DC subtypes sorted, as described in full detail elsewhere (Vremec and Segura, 2013). Cells were labelled with antibodies against CD11c(N418, used as a PE conjugate) CD45RA (14.8, used as an APC conjugate), CD8 (YTS169.4, used as a PerCp.Cy5.5 conjugate) and CD172a (P84, used as an FITC conjugate). Any residual NK cells, T cells or B cells were identified using biotinylated antibodies against CD49b (DX5), TCRβ (H57-597) and CD19 (1D3) respectively, and stained with a PE.Cy7-Streptavidin secondary reagent. Propidium iodide (PI, 0.5 μg/mL) was added in the final wash to label dead cells. Sorting was performed on a FACSAriaII instrument (BD, San Jose, CA, USA) equipped with a 100-micron aperture to reduce shear force damage to DCs, gating out dead cells, autofluorescent cells and doublets. The pDCs were sorted as CD11c^{int} CD45RA^{hi} CD49b$^-$ CD19$^-$ TCRβ$^-$, the CD8$^+$ cDCs as CD11c^{hi} CD45RA$^-$ CD49b$^-$ CD19$^-$ TCRβ$^-$ CD8$^+$ CD172a$^-$ and the CD8$^-$ cDCs as CD11c^{hi} CD45RA$^-$ CD49b$^-$ CD19$^-$ TCRβ$^-$ CD8$^-$ CD172a$^+$. Sorted cells were recovered, counted and suspended in culture medium. Purity of sorted DC subtypes was 98–99%.

2.3. Culture survival assays

The incubation medium (Naik et al., 2005; Sathe et al., 2011) was a modified RPMI-1640 medium with additional HEPES buffering, iso-osmotic with mouse serum (308 mOs), containing 10% foetal calf serum (FCS). Incubation was at 37 °C in a 10% CO2-in-air incubator. Cells were cultured at 5×10^5 cells/mL, in a total volume of 200 μL, in flat-bottom wells of a 96-well plate (BD). When required, cytokine were added at their normal optimal concentrations, namely: Fms-like tyrosine kinase 3 ligand (FL) at 200 ng/mL; granulocyte-macrophage colony stimulating factor (GM-CSF) at 1 ng/mL; interleukin (IL)-7 at 5 ng/mL; interleukin 3 (IL-3) at 10 ng/ml. To determine the viable cell count, both immediately after culture set up (the zero time value) and then after the 1–2 days of culture, 25 μL of a 106 particles/mL solution of blank–6.4 μm Sphero calibration beads (BD) was added to each well and the plate centrifuged. The supernatant was removed and the pellets resuspended in 50 μL EDTA-BSS-2% FCS containing 0.5 μg/mL PI. Samples were analysed on an LSRII instrument (BD) and files containing 5000 beads collected. The recovery of PI-excluding viable cells was calculated relative to the calibration bead count.

3. Results and discussion

3.1. DC death in culture

When individual DC subtypes are extracted from mouse spleen, purified by sorting then placed in a nutritive culture medium containing foetal bovine serum, they do not expand in numbers but show a rapid loss of viability (Figs. 1 and 2). The medium used supported the development of these DC types from bone marrow in culture (Sathe et al., 2013; Naik et al., 2005; Sathe et al., 2011), but lacked the cytokines used to drive DC development. Note that the figures depict the drop in the total recovery of viable cells compared to the original count, not the percentage of viable cells amongst those cells recovered; the latter can give false reflection of survival if cells that die disintegrate or otherwise disappear from the count. The different DC types differed in rate of cell death. Only around 25% of the pDC survived overnight (18 h) culture, although they show a half-life of around a week *in vivo* (O'Keeffe et al., 2002). Almost no pDC survived by 2 days of culture. The CD8$^+$ cDCs also showed very poor survival, with only 15% remaining after overnight culture, in contrast to an apparent half-life of 1.5 days in the spleen (Kamath et al., 2000). This turnover rate in spleen is now known to be due to limited DC division as well as loss by death (Waskow et al., 2008), so the actual death rate *in vivo* may be slower. The CD8$^-$ cDCs survived much better in culture, with around 70% surviving overnight, although this rate of death is still beyond the apparent half-life in

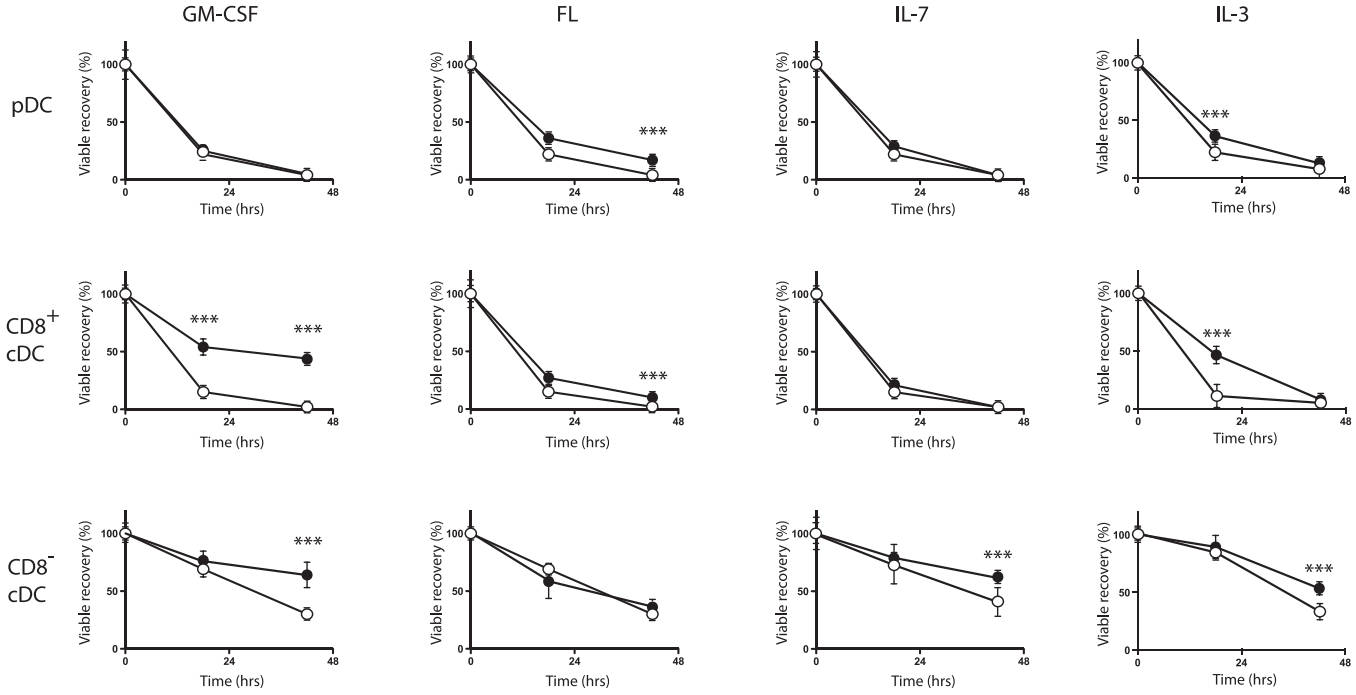

Fig. 1. The effect of cytokines on DC survival in culture. DCs were isolated and purified from pools of 16 mouse spleens, then incubated in a nutritive culture medium with or without the addition of optimal concentrations of cytokines. The total recovery of viable (PI excluding) cells is presented. Open circles indicate controls without cytokine addition, closed circles cultures with cytokine added. Results are means ± SEM of pooled data from 2 to 3 experiments, each with 5 cultures per point; SEMs may not be visible if smaller than the circle size. The significance of differences in survival is shown as *** when $p < .001$.

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