Induction of tolerogenic phenotype in human dendritic cells

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Introduction

Dendritic cells (DCs) are believed to play an important role in the maintenance of tolerance. A general consensus in the literature is that the primary function of "immature" (or some prefer to term them "semi-mature") DCs is to maintain tolerance to self, whereas "mature" DCs induce immunity.
The present study was set out to answer the following questions: (1) do immature DCs generated *in vitro* express a tolerogenic phenotype?, and (2) are there markers that can identify and distinguish functionally tolerogenic DCs from immunogenic DCs? This information is important for the currently ongoing application of immunogenic DCs in immunotherapy settings, as well as for the future application of tolerogenic DCs for treatment of autoimmune diseases in humans.

Experimental Approach

DCs were generated from peripheral blood monocytes in the presence of GM-CSF and IL-4. Briefly, monocytes were selected by plastic adherence on day 0, and were cultured in the presence of GM-CSF and IL-4 for 7 days. On day 6 of culture, some of the DCs were treated with "maturation cocktail" (TNFα, IL-1β, IL-6 and PGE_2). Tolerogenic DCs were prepared by either addition of 1,25-dihydroxyvitamin D_3 (abbreviated as **VD3**) on day 0, 3 and 5, or IL-10 (on day 5). For stability experiments (**figure 5b**) DCs on day 7 were harvested, washed extensively and re-cultured in the presence or absence of further maturation stimulus (either the same maturation cocktail or LPS). The resulting DCs were compared for their phenotypes and T cell stimulatory function.

	Day 0	Day 3	Day 5	Day 6	Day 7	Day 9/10
Immature DCs ("imDC")					Analysis / re-culture	Analysis
Mature DCs ("mDC")				+ mat. cocktail	Analysis / re-culture	Analysis
VD3-treated ("VD3 DC")	+ VD3	+ VD3	+ VD3	+/- mat. cocktail	Analysis / re-culture	Analysis
IL-10-treated ("IL-10 DC")			+ **IL-10**	+/- mat. cocktail	Analysis / re-culture	Analysis

Results

1. Do immature DCs express a tolerogenic phenotype?

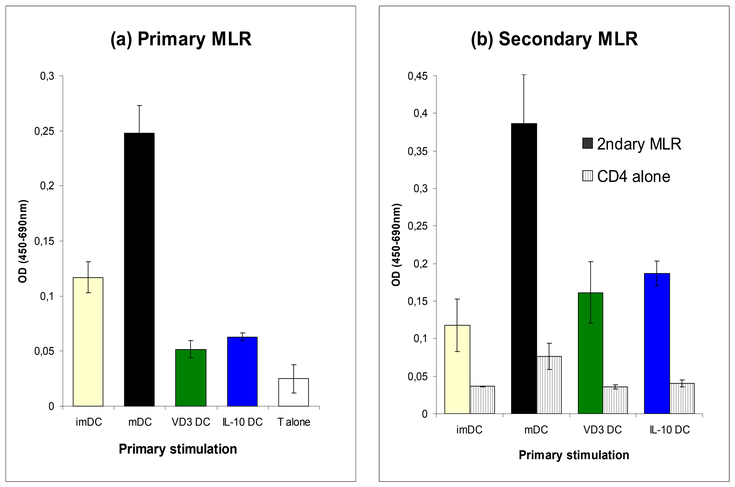


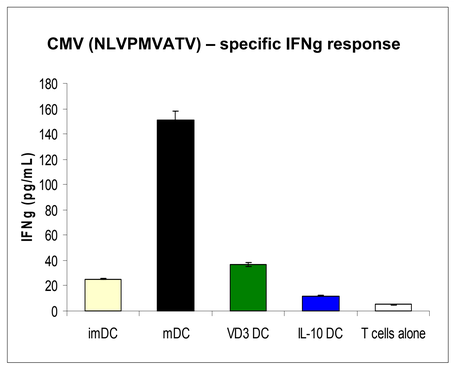
Figure 1. Both imDC and tolerogenic DC (VD3 DC or IL-10 DC) have reduced ability to induce allogeneic MLR.

(a) Primary MLR: day 7 DCs were co-cultured with allogeneic CD4+ T cells (DC:CD4 ratio of 1:10, 1:30, and 1:90. Data shown here is from the ratio 1:30) for four days. DNA synthesis was determined by measuring BrdU incorporation in the last 7 hours of incubation.

(b) Secondary MLR: CD4+ T cells from the primary MLR (as in figure 1a) was recovered by magnetic beads separation (Miltenyi Biotec) after two days of co-culture. T cells were then rested for 7-9 days in culture before they were re-stimulated by mDC of the original allogeneic donor. After two days, DNA synthesis was determined by measuring BrdU incorporation in the last 7 hours of incubation.

Figure 2. Both imDC and tolerogenic DC (VD3 DC or IL-10 DC) have reduced ability to induce CMV peptide-specific IFN-gamma production by CD8+ T cells.

Day 7 DCs were co-cultured with autologous CD8+ T cells in the presence or absence of 5uM CMV peptide (NLVPMVATV). Secreted IFN-gamma (IFNg) was measured after 24h by ELISA. Peptide specific response was calculated by subtracting the IFNg in "no peptide culture" from "+peptide culture".



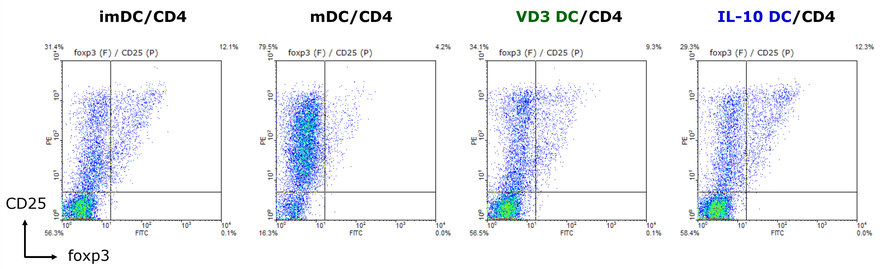


Figure 3. Preferable expansion of foxp3+ CD4+ T cells by imDC and tolerogenic DC.

T cells were collected from DC:CD4 T cell co-culture in a secondary MLR (described in figure 1b), and their surface expression of CD25 and intracellular level of foxp3 were analysed by flow cytometry.

2. Are there markers that can identify tolerogenic DCs?

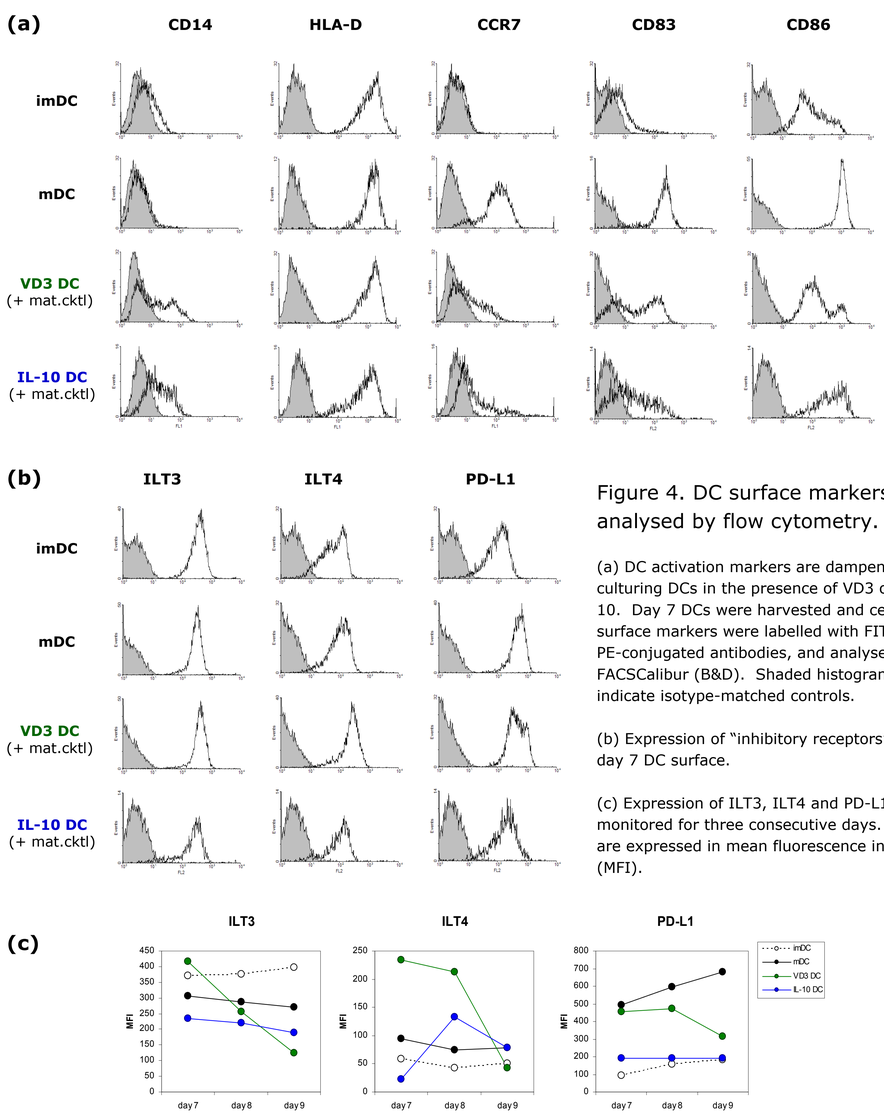


Figure 4. DC surface markers analysed by flow cytometry.

(a) DC activation markers are dampened by culturing DCs in the presence of VD3 or IL-10. Day 7 DCs were harvested and cell surface markers were labelled with FITC- or PE-conjugated antibodies, and analysed by FACSCalibur (B&D). Shaded histograms indicate isotype-matched controls.

(b) Expression of "inhibitory receptors" on day 7 DC surface.

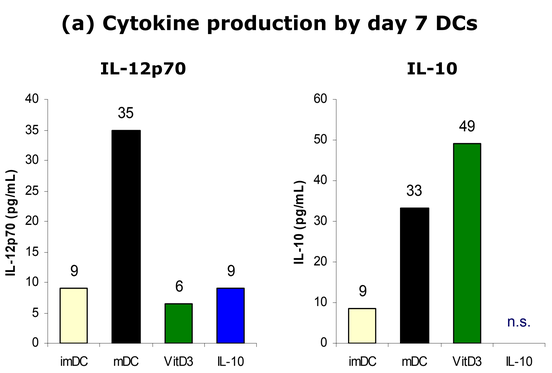
(c) Expression of ILT3, ILT4 and PD-L1 were monitored for three consecutive days. Data are expressed in mean fluorescence intensity (MFI).

Figure 5. Cytokine production by DCs.

(a) Cell culture supernatant of DCs was collected on day 7, and IL-12p70 and IL-10 were measured by standard sandwich ELISA (eBioscience).

(b) Stability experiment: DCs on day 7 were harvested, washed extensively and re-cultured in the presence or absence of further maturation stimulus. Secreted cytokine was analysed three days later (day 10).

- Day 7 DCs (before washing)
- Day 10 DCs (no further stimulation)
- Day 10 DCs (+ maturation cocktail)
- Day 10 DCs (+ LPS)



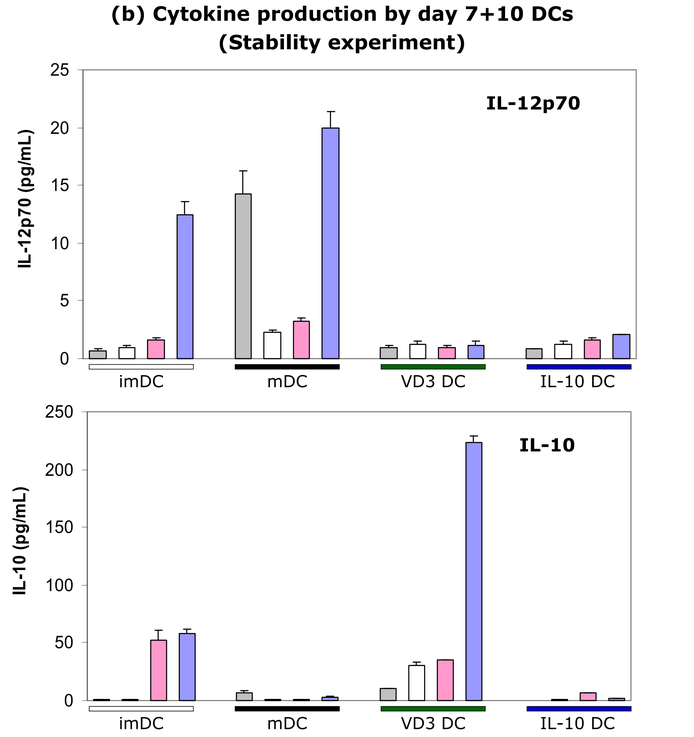
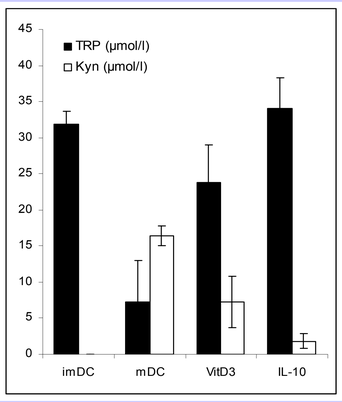


Figure 6.
IDO activity is not enhanced in tolerogenic DCs.

Tryptophan degradation via IDO was determined by evaluating kynurenine and tryptophan concentrations in supernatants of day 7 DC cultures. The values shown in the figure are means calculated from DCs derived from four separate donors.



Conclusion

Our study demonstrates that immature DCs present a tolerogenic phenotype in vitro. However, none of the markers that we examined here (ILT3, ILT4, PD-L1 or IDO) proved to be useful in identifying DCs with a tolerogenic phenotype.

It was evident that tolerogenic phenotype VD3 / IL-10 DCs was more stable than immature DCs, as the latter can produce IL-12p70 in response to further stimulation. Thus, it may be more suitable to employ *in vitro*-generated tolerogenic DCs instead of immature DCs for future application of DCs for treatment of autoimmune diseases.