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CD8 subunit expression by plasmacytoid dendritic cells is variable, and does not define stable subsets

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To the Editor: Plasmacytoid dendritic cells (pDCs) produce inflammatory cytokines upon infection with various pathogens, but can also induce production of Foxp3$^+$ regulatory T cells (T_{reg}).[1] Recently, Lombardi *et al.*[2] reported that expression of the α- and β-subunits of CD8 could be used to differentiate three stable subsets of pDC. The subsets expressing CD8α alone or in combination with CD8β were tolerogenic, inducing T_{reg} cells in a mouse model of airway hyperreactivity, whereas CD8α$^-$β$^-$pDC were immunogenic, robustly secreting inflammatory cytokines that exacerbated disease.[2] In contrast to these results, we found that CD8 expression by mature pDC is not stable but, rather, is inducible after toll-like receptor (TLR) stimulation, thereby indicating that CD8 expression does not delineate discrete and

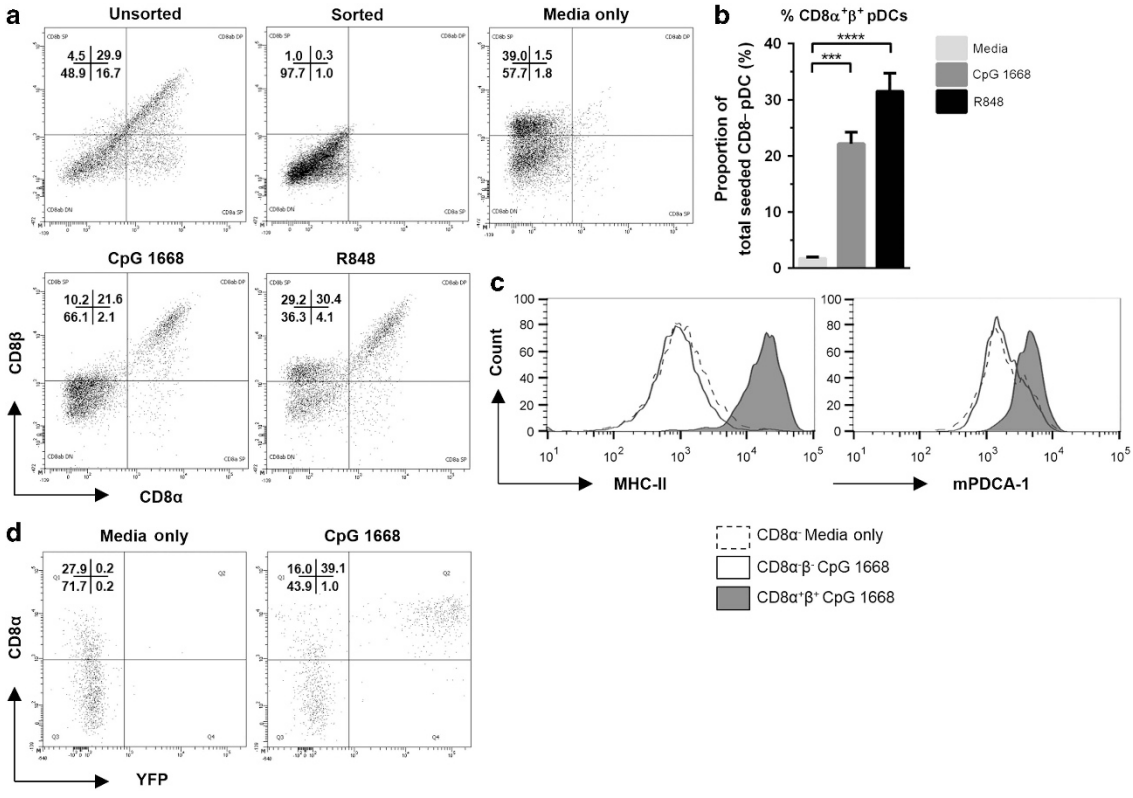

Figure 1 pDCs upregulate CD8α and CD8β upon activation *in vitro*. (**a**) pDCs were identified by flow cytometry as mPDCA-1$^+$, CD11c^{int} cells and assessed for cell surface CD8α and CD8β (unsorted). Sorted CD8$^-$ pDCs (sorted) were cultured for 16 h in media only, 0.5 μM CpG 1668, or 25 μg ml^{-1} R848, as indicated. (**b**) Proportion of pDCs that were CD8α$^+$β$^+$ after culture of CD8$^-$ pDCs as described in panel **a**. (**c**) Cell surface MHC-II (left) and mPDCA-1 (right) on CD8α$^-$ pDCs after incubation in media alone (broken line), CD8α$^-$β$^-$ pDCs (solid line), and CD8α$^+$β$^+$ (gray filled) pDCs after overnight treatment with CpG 1668. (**d**) YFP expression by semipurified pDCs isolated from IL12p40-YFP reporter mice[5] incubated in media alone or CpG 1668 as described in panel **a**. Data are representative of three independent experiments. ***$P<0.001$; ****$P<0.0001$ for proportions of CD8α$^+$β$^+$ pDCs from seeded CD8$^-$ pDCs by two-tailed Mann–Whitney *U*-test. IL, interleukin; MHC, major histocompatibility complex; pDC, plasmacytoid dendritic cell; YFP, yellow fluorescent protein.

stable pDC subsets. Purified CD8α$^-$β$^-$ pDCs were cultured in the presence or absence of TLR agonists CpG (TLR9), R848 (TLR7), or media alone. Approximately 40% of pDCs cultured in media alone expressed CD8β (**Figure 1a**). In response to either CpG or R848, a significant proportion of CD8α$^-$β$^-$ pDCs upregulated both CD8α and CD8β expression (**Figure 1a** and **b**). To determine whether the increase in expression of CD8α and CD8β was associated with cellular activation, we assessed the expression of major histocompatibility complex (MHC) class II and mPDCA-1, both of which are upregulated upon activation of pDCs.[1,3,4] CD8α$^+$β$^+$ pDCs from the cells cultured with CpG (**Figure 1c**) or R848 (not shown) had greatly elevated levels of both MHC class II and mPDCA-1 compared with CD8α$^-$β$^-$ pDCs in the same cultures or cultured in media alone. Furthermore, we found using an IL12p40-yellow fluorescent protein reporter mouse that expression of IL12p40 following CpG stimulation was restricted to CD8α$^+$ pDC (**Figure 1d**) with little interleukin (IL)12 produced directly by CD8α$^-$ pDCs (**Figure 1d**).

There have been other reports of pDCs modulating expression of CD8α upon stimulation with TLR agonists.[3,4] Therefore, we suggest that expression of CD8 subunits by pDC mirrors cellular activation rather than defining stable mature subsets with different activities. Lombardi *et al.*[2] observed differential cytokine expression by CD8-expressing pDCs. This may be because CD8-expressing pDC had been activated prior to treatment *in vitro* with agonists and thus were partially "exhausted" and unable to be fully restimulated to produce cytokines. Whether the pDC acquire a tolerogenic phenotype after their initial activation and upregulation of CD8α/β remains to be fully elucidated.

DISCLOSURE

The authors declared no conflict of interest.

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