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Reply to 'Reclassification of plasmacytoid dendritic cells as innate lymphocytes is premature'

We thank Reizis et al. for their correspondence on our Comment (Ziegler-Heitbrock, L. et al. Reclassifying plasmacytoid dendritic cells as innate lymphocytes. *Nat. Rev. Immunol.* **23**, 1–2 (2023))[1], in which we suggest that plasmacytoid dendritic cells (pDCs) with low antigen-presentation capacity and high interferon production derived from lymphoid progenitors should be assigned to the innate lymphocyte category rather than to dendritic cells (DCs) (Reizis, B. et al. Reclassification of plasmacytoid dendritic cells as innate lymphocytes is premature. *Nat. Rev. Immunol.* https://doi.org/10.1038/s41577-023-00864-y (2023))[2].

Functions of pDCs

With respect to DC function, Reizis et al.[2] argue that pDCs can undergo transcriptional remodelling and acquire antigen presentation capacity. We refer to the antigen presentation process, as executed by professional antigen-presenting cells such as DC1s and DC2s, as involving the entire process — that is, the uptake of protein antigens, their processing and intracellular loading onto MHC class II molecules, the trafficking of peptide–MHC class II complexes to the cell surface and the antigen-specific activation and proliferation of autologous T cells. One of the problems in the literature on antigen presentation in the context of pDCs is that inappropriate approaches are often used to measure this activity, such as responses of T cells to allogeneic pDCs or the external loading of pDCs with selected peptides rather than native antigens[3]. In the latter case, the pDCs do not actively process and present antigen, but simply provide cell surface MHC class II for the binding of peptides, which is a property of many cell types.

Reizis et al.[2] allude to pDC-like cells that have a similar phenotype to pDCs and which have been variably described as $CD56^+$ myeloid DCs[4], $AXL^+Siglec6^+$ DCs (AS-DCs) or pre-DCs. These pDC-like cells, which can contaminate pDC preparations, have high antigen-presentation capacity and low production of type I interferons. Thus, any contribution of these cells needs to be stringently excluded to study pure pDCs. As mentioned by Reizis et al.[2], some recent reports have attempted to address this caveat. In a study of cells generated from $LIN^-CD4^+CD11c^-AXL^-$ cells by in vitro culture[5], the population with high production of type I interferons was a poor activator of T cells. In another study, it was noted that AXL^- pDCs, after activation with CD40L and IL-3, induced a strong T cell response[6]. However, this activity of AXL^- pDCs was measured as an alloantigen response. Together, the available evidence does not demonstrate a role of pure pDCs in true antigen presentation, defined as the uptake, processing and presentation of native antigen to autologous T cells. In addition, studies on the induction of $CD8^+$ T cells by pDCs, mentioned by Reizis et al.[2], use cell preparations that have not been cleansed of pDC-like cells.

Ontogeny of pDCs

With respect to the development of pDCs and cDCs, Reizis et al.[2] mention several similarities between these two cell types and they argue that a myeloid origin of pDCs may therefore be more important than a lymphoid origin. However, there are several studies showing pDC development from $SiglecH^+FLT3^+Ly6D^+CD127^+$ common lymphoid progenitors (CLPs)[7] and the development of both $CD19^+$ B cells and pDCs from $CD127^+SiglecH^-Ly6D^+$ lymphoid progenitors[8]. In addition, on the basis of cell fate analysis, a $FLT3^+LIN^-KIT^{low}SCA1^{low}IL\text{-}7R^+$ progenitor has been identified that can give rise to both pDCs and B cells[9]. This evidence points to a substantial contribution of lymphoid progenitors to pDC production. The exact nature of such lymphoid pDC progenitors and their relationship with other progenitors of lymphoid cell populations need to be further characterized.

Reizis et al.[2] also cite several studies that have reported pDC generation under conditions that ablate the development of lymphocytes and they therefore argue for a predominant myeloid origin of pDCs. However, these early studies did not consider the existence of the phenotypically similar pDC-like cells. Therefore, it is not clear whether the cells generated from myeloid progenitors in those studies are true pDCs or instead pDC-like cells. Further studies are required to clarify whether the myeloid-derived cells with high antigen presentation capacity and low interferon production are a new type of conventional DC. In addition, the progeny of these pDC-like cells in steady state and during inflammation need to be analysed.

Together, if we agree to exclude $CD123^+CD303^+CD317^+$ pDCs with high interferon production and low antigen presentation capacity from the DC family, and when such cells are of lymphoid ontogeny, then they can be best assigned to the category of innate lymphocytes.

Activation of pDCs

Reizis et al.[2] argue that lymphocytes typically do not respond to activation by pattern recognition receptors, whereas both DCs and pDCs are triggered by Toll-like receptor (TLR) ligands, and they conclude that pDCs are therefore more compatible with a DC lineage affiliation rather than being innate lymphocytes. However, B cells can be activated via TLR7, and innate lymphoid cells (ILCs) can be activated through different TLRs; for example, ILC3s produce IL-22 when stimulated with IL-2, and ligands for TLR1, TLR2 or TLR9 (ref. 10). In any case, the type of activating molecule may not be so important; the crucial feature of pDCs is their rapid and high production of type I interferon, and this features aligns pDCs with ILCs among the innate lymphocytes.

Conclusions

We agree that future studies may add new pieces to the puzzle of pDC identity but, considering the strong functional evidence, we argue that additional pieces of the puzzle will not change the overall picture for true pDCs. Therefore, we continue to propose that pDCs should be

excluded from the DC family and that the pDCs of lymphoid origin are best assigned to the category of innate lymphocytes. Our proposal does not denigrate these cells and we want to emphasize that the CD123$^+$CD303$^+$CD317$^+$ interferon-producing cells without antigen presentation capacity are extremely important cells of the immune system.

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Competing interests

H.S. is a consultant for GlaxoSmithKline. The other authors declare no competing interests.