

## **Depletion of DC drives expansion of a unique population of monocytes poised for innate immune activation.**

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Inflammatory (Ly6C<sup>+</sup>) monocytes are an adaptable population of cells that rapidly respond to infection and tissue damage. In the absence of inflammation, mononuclear phagocyte numbers are finely controlled by access to serum growth factors. Loss of dendritic cells (DC) leads to elevated levels of Flt3L and G-CSF resulting in rapid monocytopoiesis, however, the nature of the CD115<sup>+</sup> monocytic cells that expand in the absence of DC has not been addressed.

We have exploited the CD11c-DTR model to explore homeostatic changes in the myeloid compartment following transient depletion of DC. Loss of DC results in the rapid CCR2-independent expansion of a unique population of differentiated CD64<sup>+</sup>Ly6C<sup>high</sup> monocytes. We compared the transcriptional profile of Ly6C<sup>+</sup> monocyte populations from DC-depleted or-replete mice. These data demonstrate that loss of DC results in the expansion of a monocyte-like cell that is transcriptionally distinct from other monocyte lineage populations.

We hypothesised that depletion of DC would drive expansion of monocytes ready to differentiate into monocyte-derived DC (moDC), thus refilling the DC niche. However, transfer of sorted DT-Ly6C<sup>high</sup> monocytes *in vivo* demonstrated that only a minority of these cells differentiated into CD11b<sup>+</sup>CD11c<sup>int</sup> moDC in the spleen. Rather, depletion of DC resulted in the expansion of an effector monocyte population that is primed for innate activation due to the up-regulation of the toll-like receptor sensing and signalling apparatus. Stimulation of DT-Ly6C<sup>high</sup> monocytes with the TLR4 agonist LPS resulted in the secretion of higher amounts of TNF $\alpha$  compared to Ly6C<sup>+</sup> monocytes from DC-replete controls.

In conclusion, we have demonstrated that depletion of DC drives the expansion of a unique monocyte population that is poised for immune activation, and with the potential to provoke a pro-inflammatory immune response. Studies using DC depletion models to demonstrate a requirement for DC may therefore require careful evaluation in the light of these data.