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T CELLS

# One sparrow doesn't make a summer

The kinetics and magnitude of CD8$^+$ T cell proliferation and functional differentiation are reproducible for various types of acute infection. However, two research groups now report in *Science* that this reproducibility does not reflect a homogeneous response at a single-cell level, even among monoclonal CD8$^+$ T cells.

Buchholz *et al.* and Gerlach *et al.* used two different cell fate mapping techniques to study the individual responses of naive monoclonal CD8$^+$ T cells following infection. Buchholz *et al.* adoptively transferred single naive OT-I T cells (which are CD8$^+$ T cells that are specific for the ovalbumin (OVA) epitope SIINFEKL) either with or without a population of 100 naive OT-I T cells into naive recipient mice. These mice were then infected with OVA-expressing *Listeria monocytogenes* (LM-OVA).

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Using a set of heritable congenic markers they could distinguish the fate of the OT-I T cell population from the fate of a single naive OT-I T cell. In a similar model of LM-OVA infection, Gerlach *et al.* transferred dozens to hundreds of naive OT-I T cells that had been individually marked with DNA 'barcodes' (unique DNA sequences that are passed to the daughter cells following T cell proliferation).

Analysis of the resulting CD8$^+$ T cell responses showed that just 5% of the naive OT-I T cells gave rise to 50–60% of the OT-I T cell progeny. Some of the expanded OT-I T cell families (each of which comprises all of the cells that have descended from a single naive OT-I T cell) were substantially larger than others, whereas 50% of the families were relatively small (comprising less than 200 progeny cells). Thus, single naive CD8$^+$ T cells do not respond homogeneously in the context of infection, and activation of at least 50 naive monoclonal CD8$^+$ T cells seems to be required for a robust CD8$^+$ T cell response.

Single naive CD8$^+$ T cell variability was observed not only in terms of their proliferation but also in terms of their functional differentiation: each OT-I T cell family contained a different proportion of effector and memory T cells, as defined by cell surface phenotype (in both studies), or by the cytokine and transcription factor profiles (Buchholz *et al.*). Notably, the families that showed greater expansion contained more effector and effector memory T cells, whereas small families were predominantly composed of central memory T cells. Computational modelling by Buchholz *et al.* indicated that naive T cells might differentiate linearly through a central memory precursor T cell stage into effector memory precursors and effector T cells, and that stochastic early events might determine the extent of effector differentiation.

Finally, both groups showed that whereas the proliferation and differentiation potential of single naive CD8$^+$ T cells is variable during primary infection, the response pattern of a given CD8$^+$ T cell family to a secondary or tertiary antigenic challenge can be predicted on the basis of the profile of the primary response. OT-I T cell families that had low levels of expansion and increased differentiation into central memory cells in the primary response gave rise to more robust secondary and tertiary responses compared with families that had initially shown a greater proliferative capacity and differentiation into effector and effector memory T cells.

Together, these findings indicate that the fate of single naive CD8$^+$ T cells is determined at an early stage by either random internal variation between individual naive T cells or by random (external) variation in the environmental cues that they receive. In either case, it is the average of the individual behaviours of monoclonal naive CD8$^+$ T cells that results in reproducible, robust primary and secondary CD8$^+$ T cell responses. This knowledge might help to develop vaccines that elicit more effective memory CD8$^+$ T cell immunity.

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**ORIGINAL RESEARCH PAPERS** Buchholz, V. R. *et al.* Disparate individual fates compose robust CD8$^+$ T cell immunity. *Science* 14 Mar 2013 (doi:10.1126/science.1235454) | Gerlach, C. *et al.* Heterogeneous differentiation patterns of individual CD8$^+$ T cells. *Science* 14 Mar 2013 (doi:10.1126/science.1235487)