Credit: Imagezoo/Getty

AHR-regulated genes such as *cyp1a*, *ahrra* and *cyp1c1*. *P. aeruginosa* suspensions collected from different phases of bacterial growth, which had distinct expression patterns of 3-o-C12-L-HSL and Pyo, differentially activated AHR as measured by increasing *cyp1a* expression towards later phases of bacterial growth. Similarly to the human and mouse data, 3-o-C12-L-HSL, HHQ and PQS, but not C4-L-HSL, inhibited 1-HP-induced activation of zebrafish AHR. Relative to wild-type *P. aeruginosa*, a bacterial mutant overexpressing phenazines (such as 1-HP) increased Cyp1a activity in zebrafish larvae, whereas a mutant overexpressing 3-o-C12-L-HSL decreased Cyp1a activity. Furthermore, the 3-o-C12-L-HSL-overexpressing bacteria induced a different pattern of cytokine and chemokine expression to wild-type bacteria in both wild-type and AHR-knockout mice. Whereas AHR-knockout mice infected with wild-type bacteria had fewer neutrophils in their lungs than infected wild-type mice, these differences were lost when mice were infected with 3-o-C12-L-HSL-overexpressing bacteria, which shows that the patterns of expression of QS molecules are relevant to functional immune responses.

Kaufmann and colleagues propose that this ability of AHR to detect relative levels of different *P. aeruginosa* QS molecules, both activators and inhibitors, during the course of infection allows the host to conserve energy and prevent collateral damage by only mounting an immune response when warranted by the bacterial density and stage of infection.

*Kirsty Minton*

**ORIGINAL ARTICLE** Moura-Alves, P. et al. Host monitoring of quorum sensing during *Pseudomonas aeruginosa* infection. *Science* **366**, eaaw1629 (2019)

viral reactivation in vivo. SIV-infected macaques that had received ART for at least 1 year were injected either with an antibody that depletes CD8$^+$ T cells and natural killer cells, with N-803 or with a combination of the two. Treatment with N-803 only increased viral titres in the macaques when combined with CD8$^+$ T cell depletion. Animals treated with the combination strategy showed a level of viral reactivation that was well above that observed with CD8$^+$ T cell depletion alone, or with any other LRA previously tested. However, it did not affect the frequency of latently infected CD4$^+$ T cells. The combination strategy for latency reversal was also tested in HIV-infected humanized mice, which confirmed that N-803 induces robust and persistent reversal of latency only when CD8$^+$ T cells are depleted.

The role of CD8$^+$ T cells in promoting viral latency was further investigated in vitro using autologous CD4$^+$ and CD8$^+$ T cells from uninfected donors. N-803 potently reactivated HIV expression in monocultures of latently infected memory CD4$^+$ T cells. However, this activity was significantly supressed when activated, unprimed CD8$^+$ T cells were co-cultured with the latently infected cells. These findings confirm the discovery of a previously unrecognized activity of CD8$^+$ T cells in supporting the maintenance of viral latency. How this works is currently unknown, but the authors point out that the identification of the molecular pathways that promote latency may allow their targeting without the need for CD8$^+$ T cell depletion.

Both studies demonstrate robust strategies to chase the virus out of hiding, albeit without affecting the frequency of latently infected cells. Nevertheless, they demonstrate that the first step of shock-and-kill approaches may be achievable and provide model systems to test dedicated 'kill' interventions.

*Alexandra Flemming*

**ORIGINAL ARTICLES** McBrien, J. B. et al. Robust and persistent reactivation of SIV and HIV by N-803 and depletion of CD8$^+$ cells. *Nature* https://doi.org/10.1038/s41586-020-1946-0 (2020) | Nixon, C. C. et al. Systemic HIV and SIV latency reversal via non-canonical NF-κB signalling in vivo. *Nature* https://doi.org/10.1038/s41586-020-1951-3 (2020)

# Journal Club

## MARKING TRUE $T_{FH}$ CELLS

For students in my lab who want to study T follicular helper ($T_{FH}$) cells, a paper by Jason Cyster and colleagues published in 2007 is a must-read. To help B cells to form germinal centres (GCs), T cells need to relocate into the B cell follicle and then into the GC. Although the follicular homing chemokine receptor CXCR5 had long been implicated in this process, it had not been fully characterized how exactly T cells accomplish those relocation steps. Another important question at the time was whether GC-associated T cells are different from T cells found elsewhere in the body and whether any marker could uniquely identify such cells by flow cytometry, the favorite tool of immunologists owing to its much higher processing throughput than histology or live imaging.

The study by Haynes et al. provides detailed characterization of how a combination of CXCR5 upregulation and CCR7 downregulation is important for T cells to be efficiently positioned in the follicle, and it also identifies PD1 as a marker for the CXCR5$^{hi}$CCR7$^{low}$ population of GC-associated T cells. This link between tissue location and surface phenotype greatly facilitated the further characterization of $T_{FH}$ cells, and PD1 has since become an essential part of the surface-phenotyping panel for $T_{FH}$ cells.

However, in more recent literature, the use of PD1 as a marker for $T_{FH}$ or $T_{FH}$-like cells among activated CD4$^+$ T cells in many other settings is so common that it can cause confusion. Haynes et al. were very careful to describe the context of their findings as GCs induced by immunization and GCs in the Peyer's patch. In that context, assigning PD1 as a marker for GC-associated T cells was backed up by immunohistology. In other settings, whether and how PD1 expression relates to T cell positioning or particular helper functions are questions that have to be answered on an individual basis. This is a crucial point that many students initially do not realize.

Another interesting set of observations by Haynes et al. was that T cell-specific CXCR5 ablation almost completely prevented T cells from correct positioning in the follicle, but did not prevent T cells from accessing GCs and only caused a twofold reduction in the GC response, a finding that was also reported in a separate study by Arnold et al. It remains an open question how CXCR5-deficient T cells enter GCs. What do these observations tell us about the essence of $T_{FH}$ cells, if they are defined only as CXCR5$^{hi}$PD1$^{hi}$ cells? That is also a question that students of today must think hard about.

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**ORIGINAL ARTICLE** Haynes, N. M. et al. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1$^{high}$ germinal center-associated subpopulation. *J. Immunol.* **179**, 5099–5108 (2007)
**RELATED ARTICLE** Arnold, C. N. et al. The germinal center response is impaired in the absence of T cell-expressed CXCR5. *Eur. J. Immunol.* **37**, 100–109 (2007)