HIV

Dynamic T cells

In vivo studies of T-cell homeostasis in humans have, until now, been hindered by the lack of available tools that are safe for human use. But now, reporting in *The Journal of Clinical Investigation*, Hellerstein and colleagues use a highly innovative technique to define the dynamics of T-cell proliferation *in vivo* during HIV-1 infection.

The T-cell pool can be divided into populations that are functionally and kinetically distinct: memory cells have a long life span and high proliferative capacity; by contrast, effector cells typically die quickly by activation-induced cell death. The size of the T-cell pool is mainly regulated and maintained by proliferation of long-lived progenitor cells. In patients infected with HIV, a shortened average life span of T cells has been previously described. However, it remains controversial whether this results from direct cell killing by HIV or indirect effects of chronic activation. Without the benefit of cell-type specific markers that distinguish long- and short-lived cells, Hellerstein *et al.* developed an alternative approach to characterize the variation in life span in the memory/effector T-cell pool. By administration of deuterium — a safe, stable, non-radioactive isotope of hydrogen — in the form of deuterated water and deuterated glucose, which is incorporated into the DNA of dividing cells, the authors were able to measure T-cell kinetics *in vivo* over long periods of time. Long-term incorporation of deuterated water into DNA indicated that effector/memory T-cell subpopulations, but not naive T cells, had biphasic kinetics, consistent with the presence of T-cell subpopulations in humans with different life spans. This technique also enabled the detection in normal individuals of long-lived quiescent T cells that did not divide over the 9 week deuterium administration, probably representing the reservoir of progenitor cells.

They then went on to show that individuals with advanced HIV-1 infection had higher proportions of T cells that were short lived, in both the $CD4^+$ and $CD8^+$ memory/effector T-cell subpopulations, compared with healthy controls. These results were confirmed by analysis of die-away kinetics after short-term labelling with deuterated glucose, and together with long-term kinetic analysis, pool sizes and turnover rates of kinetically distinct subpopulations of T cells were calculated. In advanced HIV-1 infection, total pool sizes of short-lived cells were only moderately affected, whereas the

SIGNALLING

New link in the chain

After recognition of microbial products by members of the Toll-like receptor (TLR) family, the adaptor molecule MYD88 initiates intracellular signalling cascades that result in the activation of host defence mechanisms. However, recent studies have shown a MYD88-independent signalling pathway downstream of TLR3 and TLR4 that is regulated by the adaptor molecule TRIF (also known as TICAM1) and leads to the activation of interferon regulatory factor 3 (IRF3), as well as signals that sustain activation of nuclear factor-κB (NF-κB). Now, three independent studies have identified TRAM (also known as TICAM2) as an adaptor molecule that is a key link in the TRIF-dependent TLR4, but not TLR3, signalling cascade.

Previously identified TLR adaptor molecules contain Toll–interleukin 1 receptor (TIR) domains and each of the three groups identified TRAM using database searches for new TIR-domain-containing molecules. Oshiumi *et al.* and Fitzgerald *et al.* used a yeast two-hybrid assay and/or co-immunoprecipitation studies to show that TRAM interacts directly with both TRIF and TLR4, but not TLR3, implicating TRAM as a specific component of the TLR4-signalling cascade.

To determine the role of TRAM in TLR4 signalling, all three groups used the same approach — analysing the effect of ectopic overexpression of TRAM — and all observed activation of IRF3 and NF-κB, as well as activation of the promoter for the gene encoding interferon-β (IFN-β).

Oshiumi *et al.* and Fitzgerald *et al.* went on to generate a series of TRAM mutants, which they used to show that TRAM is crucial for IRF3 and NF-κB activation after ligation of TLR4 by lipopolysaccharide (LPS), but not TLR3 triggering with double-stranded RNA. Further evidence for the importance of TRAM in the TLR4-signalling pathway was provided by their demonstration that marked reduction of the level of TRAM by small interfering RNA impaired IRF3 and NF-κB activation in response to TLR4 triggering, but not TLR3 ligation.

Yamamoto *et al.* examined the physiological role of TRAM in the TLR-signalling cascade by generating Tram-knockout mice. Tram-deficient cells responded normally to signalling through TLR3; however the production of pro-inflammatory cytokines and IFN-β was substantially impaired after LPS triggering of TLR4, as was the activation of B cells. These functional defects coincided with inefficient activation of signalling molecules downstream of TRAM, including IRF3, and an inability to sustain NF-κB activation. By contrast, activation of the MYD88-signalling pathway was intact in the Tram-deficient cells. So, *in vivo,* TRAM is essential for the MYD88-independent signalling cascade after LPS ligation of TLR4. This cascade induces B-cell activation and the production of IFN-β; however, TRAM and MYD88 signals are required for the secretion of pro-inflammatory cytokines in response to LPS.

These studies provide invaluable insight into the TRIF-regulated MYD88-independent response generated after triggering of both TLR3 and TLR4. Ligation of these molecules induces distinct host responses and this specificity might be a result of TLR4 using the adaptor molecule TRAM to initiate signalling through TRIF, whereas TLR3 signals directly to TRIF.

Karen Honey

References and links

ORIGINAL RESEARCH PAPERS Yamamoto, M. *et al*. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nature Immunol.* 13 October 2003 (DOI:10.1038/ni986) | Fitzgerald, K. A. *et al*. LPS–TLR4 signaling to IRF-3/7 and NF-κB involves the Toll adaptors TRAM and TRIF. *J. Exp. Med.* **198**, 1043–1055 (2003) | Oshiumi, H. *et al*. TICAM-2: a bridging adaptor recruiting to Toll-like receptor 4 TICAM-1 that induces interferon-β. *J. Biol. Chem.* 30 September 2003 (doi:10.1074/jbc.M305820200)