infection. CD8αα$^+$ and CD8αα$^-$ P14 fractions were then transferred separately to naive recipients, and the mice were challenged with LCMV 40 days later. Only those mice that had received the CD8αα$^+$ fraction had a significant memory P14 T-cell response to the virus, and their T cells produced interferon-γ *in vitro* when stimulated with LCMV peptide. An enhancer deletion in the mouse *Cd8* locus that prevents the high-level expression of CD8α required to form CD8αα homodimers, but still allows CD8αβ heterodimer formation, did not affect the primary response to LCMV but led to low numbers and a poor *in vitro* response of LCMV-specific T cells 50 days after infection, which confirms the essential role of CD8αα in T-cell survival and memory-cell differentiation.

Kirsty Minton

References and links

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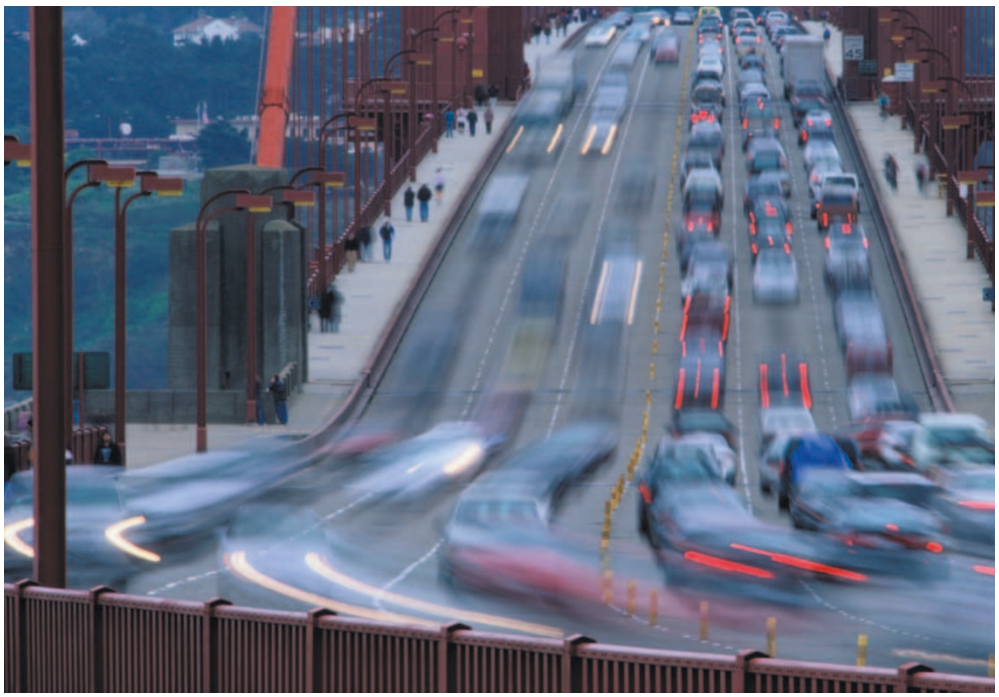

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Destroying Tolls

Toll-like receptor (TLR) signalling generates a pro-inflammatory immune response that is capable not only of clearing infection but also of damaging host tissues. It is therefore important to tightly regulate the TLR-induced response, and a paper published recently in *Nature Immunology* describes a novel mechanism for doing just that — TLR ubiquitylation and targeting for degradation.

Ubiquitin targeting of proteins for proteolytic degradation is a three-step process. First, ubiquitin is activated by a ubiquitin-activating enzyme (E1); second, activated ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2 or UBC); and third, an E3 ubiquitin ligase attaches ubiquitin to lysine residues on the protein to be targeted for degradation.

Chuang and Ulevitch reasoned that the cytoplasmic Toll/interleukin-1-receptor (TIR) domain of TLRs, which is crucial for recruiting proteins involved in TLR signalling, might also bind proteins involved in negatively regulating TLRs. Using a yeast two-hybrid approach, they found that the TIR domain of human TLR9 binds a previously described protein known as TRIAD3. This interaction was not specific for the TIR domain of TLR9, as TRIAD3 also interacted with TLR3, TLR4 and TLR5, but it did not interact with TLR2.

Further analysis of TRIAD3 identified five splice variants, designated TRIAD3A–E, of which TRIAD3A was the most ubiquitous and abundant. TRIAD3A was predicted to contain RING motifs characteristic of the RING-finger group of E3 ligases. Consistent with this, TRIAD3A was shown to interact with some E2 enzymes — UBCH7 and to some extent UBCH8, but not with UBCH2 or UBCH10. Furthermore, when overexpressed in the 293 cell line, TRIAD3A induced auto-ubiquitylation, as well as ubiquitylation of TLR9 but not TLR2.

In separate studies, TRIAD3A promoted degradation of TLR9 and TLR4, but had no effect on the level of TLR2. TRIAD3A was shown to function in a dose-dependent manner, and degradation of TLRs was abrogated in the presence of a proteasome inhibitor but not by inhibitors of lysosomal proteolysis. The decrease in TLR protein levels was concomitant with reduced signalling in response to stimulation with TLR4 and TLR9 ligands, whereas TLR2 ligands induced a normal signalling response.

Together, these data indicate that TRIAD3A is an E3 ligase that regulates the ubiquitylation of specific TLRs, targeting them for proteasomal degradation. Further evidence for this was provided by the observation that TRIAD3A-specific small interfering RNAs (siRNAs), which substantially reduce the expression of TRIAD3A protein, increased the basal level of TLR9 expression, and abolished the decrease in TLR9 expression that is normally observed after stimulation with TLR9 ligands. Similarly, TRIAD3A-specific siRNAs induced increased activation after exposure to TLR4 ligands but not TLR2 ligands.

This study identifies a novel mechanism for negative regulation of specific TLR-signalling pathways — TRIAD3A-regulated ubiquitin-dependent proteasome degradation. Future studies will no doubt focus on how TRIAD3A is recruited to the TLR-signalling complex to mediate its specific regulatory effects.

Karen Honey

References and links

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