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INNATE IMMUNITY

The inside story on complement activation

The evolutionarily ancient complement system might have started life as an intracellular activation pathway rather than as a liver-derived serum effector cascade. Other recent studies have suggested the importance of T cell-derived local complement activation in the autocrine stimulation of T helper 1 (T_H1) cell responses, but Claudia Kemper, John Atkinson and colleagues are the first to show that the C3 activation products C3a and C3b can be generated intracellularly in human $CD4^+$ T cells.

Activation of mouse T cells is thought to induce the expression of C3, factor B and factor D proteins, resulting in extracellular assembly of a C3 convertase and cleavage of C3. However, the rapid generation of C3a and C3b by human T cells is inconsistent with *de novo* protein production, so the authors looked for a potential role of endogenous proteases. They showed that resting human T cells express mRNA encoding cathepsin L (CTSL), and that CTSL cleaves C3 into biologically active C3a and C3b fragments *in vitro*. C3 and CTSL were found to be colocalized in resting T cells in the endoplasmic reticulum (ER) and ER-derived secretory vesicles.

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Intracellular C3a was detected in resting T cells, and the increase in intracellular C3a levels observed after antibody-mediated T cell activation could be prevented by a cell-permeable CTSL inhibitor. C3a, C3b, CTSL and C3a receptor (C3aR) were present on the surface of activated but not resting T cells, and C3a levels on the T cell exterior could be decreased both by the cell-permeable CTSL inhibitor and by a cleavage-blocking CTSL-specific antibody. So, the data indicate that a system for ‘tonic’ intracellular C3a generation by CTSL in resting T cells is both upregulated and translocated to the cell surface after T cell activation.

CTSL inhibition in resting and activated T cells decreased mammalian target of rapamycin (mTOR) phosphorylation and cell survival, as did the inhibition of intracellular C3aR expression or signalling. Cell viability could not be rescued by extracellular C3a supplementation, which indicates that intracellular C3a–C3aR signalling is required for T cell survival. Further studies showed that cell surface C3aR engagement after T cell activation drives effector function. When T cells were activated in the presence of a low concentration of CTSL inhibitor — which decreases cell surface C3a levels without affecting cell viability — the production of interferon-γ (IFNγ) and interleukin-17 was significantly decreased but could be partially rescued by the addition of C3a. Cytokine production was not affected in serum-free medium or in the presence of a C3 convertase inhibitor, which shows that the CTSL-mediated C3 activation pathway is sufficient for autocrine C3aR-induced T cell effector function in humans.

The *in vivo* relevance of this pathway was shown in synovial fluid $CD4^+$ T cells from patients with juvenile idiopathic arthritis. The T cells had increased levels of intracellular C3a, mTOR activation and IFNγ production compared with peripheral blood T cells from the same patient or from a healthy donor. The addition of a CTSL inhibitor to patient synovial T cell cultures ‘normalized’ all of these parameters in a dose-dependent manner. So, the uncontrolled T cell activity in this disease might be due, at least in part, to the deregulation of CTSL-mediated C3a generation.

This demonstration of intracellular complement activation has important implications for how the complement pathway might interact with other components of innate immunity, such as intracellular pattern recognition receptors. Furthermore, cells of myeloid, lymphoid and non-myeloid, non-lymphoid origin were all shown to generate intracellular C3a, so the authors suggest that intracellular complement activation might be a general phenomenon with potential roles in other basic cell processes.

Kirsty Minton

ORIGINAL RESEARCH PAPER Liszewski, M. K. *et al.* Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity* **39**, 1143–1157 (2013)