INFLAMMATION

Dying cell-derived SAM switches off inflammation

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The clearance of dying cells by macrophages releases mediators that switch off inflammation. Ampomah *et al.* now reveals how an amino acid derived from apoptotic cargo changes the methylation epigenome of macrophages to take the brakes off an Erk-dependent pathway that terminates inflammatory responses.

Inflammation is characterised by an initial onset phase, which is an active process that drives the cardinal signs of heat, redness, swelling and pain. Ultimately, when the inciting stimulus has been eliminated, onset then progresses towards the equally active phase of resolution[1]. However, unlike onset, which has been well characterised over the years, resolution is comparatively less well understood. With the challenge of uncovering new biological pathways underpinning health and well-being that will inform on the aetiology of chronic diseases, to the logical progression towards developing drugs based on harnessing these internal "off-switches", resolution of inflammatory process holds much excitement and promise.

Resolution is a crucial aspect of inflammation, as it clears cellular debris, alters the balance of soluble inflammatory compounds, and generally invokes tissue homeostasis. Moreover, we know that resolution is crucial in setting up optimal environments for continued, adaptive immune responses[2-7]. A key step in switching from inflammatory onset to resolution is the process of phagocytic, non-phlogistic clearance of dying cells – efferocytosis[8].

How efferocytosis mechanistically drives inflammatory resolution is poorly understood. However, work has shown that efferocytosis results in phenotypic shifts in macrophages thereby changing mediator profiles. The release of two of these key mediators, prostaglandin

E_2 (PGE_2) and transforming growth factor beta (TGFβ)[9], has been known to be important for some time, but the molecular mechanisms orchestrating this have not. Now, the work by Ampomah *et al.* in this issue of Nature Metabolism shows that apoptotic cell-derived methionine is used by phagocytes to induce DNA-methyltransferase-3A activity promoting pro-resolving pathways ultimately leading to the release of PGE_2 and TGFβ (REF Ampomah).

Using multiple approaches in both human and mouse-derived cells, the authors show that the ingestion of apoptotic cells (ACs) by macrophages results in activation of the Erk signaling pathway, predominantly by AC binding to CD36. CD36 is a scavenger receptor involved in efferocytosis as well as binding ligands such as collagen, oxidized phospholipids and thrombospondin[10,11]. CD36-dependent Erk activation induces expression of *Ptgs2*, the gene encoding for Cyclo-oxygenase 2 (COX2). This enzyme produces prostanoids, key amongst which is PGE_2. PGE_2 has pleiotropic functions but plays a role in resolution[12]. Here, the authors show that auto-/paracrine action of PGE_2 results in the production and release of TGF-β. Together these molecules act to facilitate resolution and wound healing.

However, the authors show that stimulation of CD36 alone achieves insufficient Erk activation to orchestrate these events. Tonic activity of the phosphatase Dusp4 prevents excessive Erk signaling. Using heavy ^{13}C-^{15}N-methionine labelled ACs, the authors show that macrophage methionine adenosyltransferase 2A (MAT2A) converts phagolysosomal-derived methionine into S-adenosylmethionine (SAM). SAM allows DNA-methyltransferase-3A (DNMT3A) to rapidly methylate certain genomic loci, including that encoding for Dusp4. This effectively represses Dusp4 function allowing for uninterrupted Erk signaling and PGE_2/TGF-β production, **see Figure**.

Thus, the authors provide a novel way in which AC ingestion can alter macrophage function, by epigenetic regulation via DNA methylation. This adds to our knowledge on how AC-derived metabolite pathways govern macrophage function. Previously, others have shown how these pathways can result in altered macrophage energy metabolism or transcription[13,14]. Of course, the authors draw attention to this singular methylation change; others have yet to be described in what will surely be highly exciting future publications.

Intriguingly, the presence of methionine in the culture media was not required to achieve DNMT3A activation in macrophages, suggesting that exogenous addition or alteration of methionine levels would not be a viable therapeutic intervention. The evidence presented by the authors highlights the crucial role of the phagolysosome in repressing Dusp4. Furthermore, the need to obtain methionine from phagolysosomal degradation hints at an activation threshold for Dusp4 repression to potentially prevent aberrant or premature termination of pro-inflammatory pathways.

Finally, the authors finished with compelling *in vivo* work using DNMT3A knockout mice and multiple models of inflammation with prominent efferocytosis components: 1) Dexamethasone-induced thymus injury modelling continuous removal of apoptotic thymocytes by thymic macrophages (non-inflammatory); 2) Zymosan peritonitis, acute removal of apoptotic neutrophils infiltrated post-sterile inflammation (inflammatory); 3) Atherosclerosis using LDLR knockout mice transplanted with DNMT3A knockout bone marrow, focusing on the role of TGF-b in stabilising atherosclerotic plaques.

All three models elegantly displayed the importance of DNMT3A in orchestrating efferocytosis and preventing debris build up and chronic inflammation. The latter model is particularly apt as treating with the aim to regress atherosclerosis remains a clinical challenge. While it's been suggested that manipulating efferocytosis is a tractable strategy to resolve atherosclerosis[15], the pathway presented here by Ampomah *et al*. (ref) could provide novel avenues of treatment. Ultimately, the SAM-DNMT3A-PGE_2-TGF-β1 axis provides multiple putative therapeutic targets to be explored in future.

However, the precise mechanism of action of DNMT3A in bringing about pro-resolving release of PGE_2 and TGF-β is still unclear. Firstly, the authors show that other types of phagocytic cargo (opsonised erythrocytes) might also induce this pathway. Thus, more work will need to look into exactly what phagolysosomal pathways are involved in DNMT3A activation and which are not. Here, the authors show the role of LC3-associated phagocytosis (LAP) in promoting DNMT3A activation, but it remains to be seen if this is specific to AC- or opsonised cargo, or to LAP in general. Furthermore, the overall epigenetic changes as a result of DNMT3A activation are unknown and could extend far beyond the activation of COX-2/TGF-β. Indeed, one does wonder how susceptible this system is to variations in methionine levels of the ingested cargo. Would infected ACs produce a similar epigenetic profile as non-infected ACs? Does opsonisation alter methylation sites? These and other questions will need to be addressed in future.

There is much we have yet to discover about inflammatory resolution. But increasingly, we are learning that the interaction of ACs with phagocytes can dictate immune outcome, including the ligation of different efferocytosis receptors on macrophages and the signalling complexes linked to them, the method of phagocytosis and lysosomal maturation, and the use of AC-derived breakdown products. Now, the epigenetic regulation of macrophages by ACs. All these different elements are crucial, and we are only at the start of our journey towards understanding how they combine to orchestrate inflammation. It is a worthwhile journey, however, as the ability to harness resolution biology may well be the vital link in novel treatment approaches in diseases characterised by chronic and/or overblown inflammation such as atherosclerosis, systemic lupus erythematosus, and sepsis.

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Conflicts of interest

The authors declare no competing interests.

Figure legend: Initial uptake of apoptotic body *via* CD36 drives pro-resolution/healing TGFβ secretion, which is blocked, in a negative feedback manner, by DUSP4. However, during subsequent efferocytosis DUSP4 induced negative feedback is lifted upon subsequent efferocytosis whereby apoptotic body-derived methionine is converted into s-adenosylmethionine, which represses DUSP4. (-ve denotes negative effect/blocking a pathway while +ve = positive/driving a pathway).