Nature Reviews Immunology | Published online 29 Feb 2016; doi:10.1038/nri.2016.23

S. Bradbrook/NPG

CELL DEATH

Find me and eat me

Phagocytic removal of apoptotic cells in an immunologically silent way is important to maintain tissue homeostasis; failures in this process can lead to inflammatory diseases and autoimmunity. Reporting in *Immunity*, Luo *et al.* show that the release of sphingosine-1-phosphate (S1P) from dying cells activates erythropoietin signalling in macrophages, which promotes apoptotic cell clearance and immune tolerance.

'Eat-me' signals, such as S1P, are exposed or released by apoptotic cells to trigger phagocytic uptake, which in turn activates tolerogenic pathways to prevent immune responses against self-antigens. The authors investigated whether erythropoietin — which can suppress inflammatory gene expression in macrophages — could have a role in the clearance of dying cells and the tolerogenic responses in macrophages.

Incubation of peritoneal macrophages with S1P or apoptotic cell-conditioned media led to increased levels of erythropoietin and erythropoietin receptor (EPOR) expression in a time-dependent manner. Further experiments *in vivo* showed that intravenous administration of apoptotic Jurkat cells led to increased levels of erythropoietin in the spleen and increased expression of EPOR by splenic macrophages, whereas this effect was abolished after administration of apoptotic Jurkat cells in which sphingosine kinase 1 had been silenced by small interfering RNA. Increased expression of erythropoietin and EPOR was also seen when thymocyte apoptosis was induced endogenously by dexamethasone, but this effect was reduced when the S1P receptor was inhibited. Furthermore, suppression of cell apoptosis *in vivo* reduced the levels of erythropoietin and EPOR expression in macrophages. Thus, both exogenously given and endogenously induced dying cells increased the expression of erythropoietin and EPOR in a S1P-dependent manner.

Next, the authors investigated whether macrophage erythropoietin signalling is involved in apoptotic cell clearance. *In vitro* experiments showed that *Epor*$^{-/-}$ peritoneal macrophages had a ~54% reduction in apoptotic cell phagocytosis compared with wild-type cells. This effect was also seen in mice with a conditional deletion of *Epor* in macrophages (*Epor*$^{loxp/loxp}$*Lyz2*–Cre mice), and pre-treatment with recombinant human erythropoietin promoted phagocytosis of dying cells by peritoneal or splenic macrophages in wild-type mice but not in *Epor*$^{loxp/loxp}$ *Lyz2*–Cre mice. In addition, they found that erythropoietin signalling in macrophages was important for inducing transforming growth factor-β expression and reducing tumour necrosis factor and interleukin-6 expression. Hence, macrophage erythropoietin signalling is important for apoptotic cell removal in an immunologically silent way.

Further analysis revealed that erythropoietin increased the expression of peroxisome proliferator-activated receptor-γ (PPARγ) in a time- and dose-dependent manner and deletion of *Epor* led to reduced levels of PPARγ in peritoneal macrophages. Addition of a PPARγ agonist, but not recombinant human erythropoietin, significantly enhanced phagocytosis of apoptotic cells in EPOR-deficient peritoneal macrophages *in vitro*, whereas neither of these compounds increased phagocytosis in PPARγ-deficient macrophages. Hence, erythropoietin seems to enhance dying cell clearance by upregulating PPARγ in macrophages.

Finally, the authors found that mice with EPOR-deficient macrophages developed age-dependent lupus-like disease, which could be improved by pharmacological activation of erythropoietin signalling. Interestingly, recombinant human erythropoietin has been shown to improve outcomes of autoimmune diseases but the mechanism for this is unclear. Thus, this study identifies an important role for erythropoietin signalling in macrophage clearance of dying cells, which could potentially have implications for treatment of autoimmune disease.

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> “mice with EPOR-deficient macrophages developed age-dependent lupus-like disease”

ORIGINAL ARTICLE Luo, B. *et al.* Erythropoeitin signaling in macrophages promotes dying cell clearance and immune tolerance. *Immunity* http://dx.doi.org/10.1016/j.immuni.2016.01.002 (2016)
FURTHER READING Poon, I. K. H. *et al.* Apoptotic cell clearance: basic biology and therapeutic potential. *Nat. Rev. Immunol.* **14**, 166–180 (2014)