


 IMMUNOTHERAPY

# Stopping monocytes in their tracks

Inflammatory monocytes are rapidly recruited to sites of inflammation, but their excessive and/or prolonged recruitment hinders the resolution of inflammation and is a hallmark of numerous diseases. Therefore, targeting these cells could be of therapeutic value. Reporting in *Nature Biotechnology*, Leuschner *et al.* describe a method for specifically targeting inflammatory monocytes, and this approach was shown to attenuate disease progression in mouse models of myocardial infarction, cancer, atherosclerosis and pancreatic islet transplantation in diabetes.

Inflammatory monocytes (LY6C<sup>hi</sup> in mice and CD14<sup>++</sup>CD16<sup>-</sup> in humans) give rise to pro-inflammatory macrophages and express high levels of CC-chemokine receptor 2 (CCR2), whereas non-inflammatory monocytes are CCR2<sup>low</sup>. *Ccr2*<sup>-/-</sup> mice have decreased levels of inflammation in numerous disease models. Therefore, the authors speculated that therapeutic targeting of CCR2 could selectively inhibit inflammatory monocyte recruitment and dampen detrimental inflammation.

To do this, the authors developed a lipid nanoparticle that encapsulated a short interfering RNA (siRNA) that targets *Ccr2* mRNA (termed siCCR2). By labelling the siRNA with a near-infrared fluorochrome, siCCR2 could be tracked in mice using fluorescence molecular

“ therapeutic siRNA-mediated silencing of inflammatory monocyte migration is a potential strategy for the treatment of numerous inflammatory diseases ”

tomography. Following intravenous injection, siCCR2 was rapidly cleared from the blood and accumulated in the spleen and bone marrow (sites of monocyte reservoirs). In the spleen, the highest uptake of siCCR2 was by LY6C<sup>hi</sup> monocytes, although it was also taken up by other leukocytes. Treatment with siCCR2 resulted in decreased CCR2 expression on splenic LY6C<sup>hi</sup> monocytes, and these cells did not migrate towards CCL2 in a migration assay.

So, the siRNA can reach and knock down its target, but does this approach have any therapeutic benefits? To test this, the authors used several different models of diseases that are known to be associated with high levels of monocyte recruitment. In the first model, pre-treatment of mice for 3 days with siCCR2 prior to the induction of ischaemia–reperfusion injury resulted in reduced numbers of monocytes and macrophages in the heart and reduced the infarct size by 34%. Interestingly, the beneficial effects of siCCR2 were preserved when it was administered one hour after injury. By removing the spleen at the time of injury, the authors showed that the main target of siCCR2 in acute inflammation is the splenic monocyte reservoir.

Similarly, treatment of mice that have atherosclerosis (apolipoprotein E-deficient (*ApoE*<sup>-/-</sup>) mice) for 3 weeks

with siCCR2 significantly reduced the number of LY6C<sup>hi</sup> monocytes in the atherosclerotic plaques and reduced the lesion size in the aortic root. Furthermore, siCCR2 treatment of mice with diabetes (induced by streptozotocin) 3 days prior to pancreatic islet transplantation significantly extended the normoglycaemic period — which is indicative of prolonged graft survival — compared with control mice. Finally, siCCR2 treatment of mice that had palpable tumours following implantation of lymphoma EL4 cells resulted in a reduction in tumour growth, a 54% reduction in tumour-associated macrophage numbers, a decrease in the levels of vascular endothelial growth factor and a reduction in the number of microvessels in the tumour.

So, therapeutic siRNA-mediated silencing of inflammatory monocyte migration is a potential strategy for treating numerous inflammatory diseases without disrupting inflammation resolution, which is associated with non-inflammatory monocytes and alternatively activated macrophages.

Olive Leavy

**ORIGINAL RESEARCH PAPER** Leuschner, F. *et al.* Therapeutic siRNA silencing in inflammatory monocytes in mice. *Nature Biotech.* 9 Oct 2011 (doi:10.1038/nbt.1989)

**FURTHER READING** Shi, C. & Pamer, E. G. Monocyte recruitment during infection and inflammation. *Nature Rev. Immunol.* 11, 762–774 (2011)