

 TRANSPLANTATION

## Targeting trained immunity

“macrophages are required for transplant acceptance following mTORi-HDL nanoimmunotherapy”

Successful organ transplantation depends on long-term immunosuppression, which has considerable toxicity and detrimental effects. A new study shows that targeting myeloid cell activation using nanobiologics prevents the inflammatory responses that drive graft rejection and promotes long-term immunological tolerance.

Recent work identified a crucial role for innate immune cells in initiating allograft rejection. So Braza et al. investigated the pathways that might activate graft-infiltrating macrophages and induce epigenetic reprogramming associated with ‘trained immunity’. Using an experimental transplantation mouse model, they observed an upregulation of the damage-associated molecular patterns vimentin and high mobility group box 1 (HMGB1) in allogeneic heart transplants, and graft-infiltrating monocytes expressed receptors for these ligands, dectin 1 and Toll-like receptor 4 (TLR4). Recipient mice lacking dectin 1 and TLR4 did not accumulate graft-infiltrating inflammatory Ly6C<sup>hi</sup> macrophages and instead accumulated Ly6C<sup>low</sup> macrophages, which have

been reported to promote graft tolerance. Indeed, priming purified monocytes with vimentin and then restimulating with HMGB1 led to marked production of the pro-inflammatory cytokines tumour necrosis factor (TNF) and IL-6, which is a feature of trained immunity. Moreover, expression of dectin 1 and TLR4 was shown to be necessary to establish the features of trained immunity, including epigenetic changes at the *Tnf* and *Il6* loci and ability to produce glycolytic products and pro-inflammatory cytokines. This suggested that monocytes infiltrating the allograft early after transplantation become trained on vimentin and HMGB1 exposure.

To prevent the induction of trained macrophages, the authors designed a nanobiologic comprising the mechanistic target of rapamycin (mTOR) inhibitor rapamycin encapsulated in high-density lipoprotein (HDL), termed mTORi-HDL. Imaging of fluorescently labelled or radiolabelled mTORi-HDL, given intravenously to recipient mice, revealed an accumulation of nanoparticles in the heart allograft, as well as in the blood and spleen,

with preferential uptake by myeloid cells. Importantly, three intravenous doses of mTORi-HDL were sufficient to induce a marked decrease in graft-infiltrating innate immune cells and prolong graft survival, compared with oral rapamycin or placebo controls. Moreover, mTORi-HDL-treated recipients had more Ly6C<sup>low</sup> macrophages than control recipients, and these cells showed compromised ability to produce pro-inflammatory cytokines after stimulation *ex vivo*, suggesting that macrophage training was inhibited.

Further analysis of the Ly6C<sup>low</sup> macrophages revealed that they inhibited T cell proliferation and promoted regulatory T cell expansion, which was consistent with the observed increase in CD4<sup>+</sup>CD25<sup>+</sup> T cells in allografts of mTORi-HDL-treated recipients. Depletion of Ly6C<sup>low</sup> regulatory macrophages on the day of transplantation resulted in early transplant rejection despite mTORi-HDL treatment. Allograft survival could be restored in these animals by adoptive transfer of wild-type monocytes, suggesting that macrophages are required for transplant acceptance following mTORi-HDL nanoimmunotherapy.

Finally, short-term treatment with a combination of mTORi-HDL and a second nanobiologic that inhibits CD40 co-stimulation (TRAF6i-HDL) synergistically promoted organ transplant acceptance and long-term survival. Thus, this study identifies a new treatment approach that impedes myeloid cell training and co-stimulation to promote indefinite immune tolerance.

Lucy Bird



Credit: Peter Macdiarmid/Getty

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