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Monocyte-Derived Macrophages: The Missing Link in Organ Transplantation

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Successful organ transplantation requires an optimal innate immune response to avoid tissue injury. In this issue of *Immunity*, Braza et al. (2018) inhibit pro-inflammatory activation of infiltrating graft macrophages using nanotechnology tools to promote immune tolerance, leading to long-term transplant acceptance in mouse models.

Despite significant success, allograft rejection and chronic immunosuppression after organ transplantation remain important clinical issues. Release of danger-associated molecular patterns (DAMPs) that trigger an inflammatory response combined with activation of $CD4^+$ Th1 cells leads to the generation of alloreactive $CD8^+$ T cells that mediate rejection. Myeloid cells were thought to contribute to this process through their antigen presentation capabilities, but recent work indicates that the innate immune response can set the trajectory of the initial inflammatory response through mechanisms beyond adaptive immunity.

Sterile inflammation occurs rapidly in the setting of tissue injury, whether it be due to surgical trauma during transplantation followed by ischemia reperfusion injury (IRI) or by IRI alone following a myocardial infarction. Injury leads to activation of $Ly6C^{hi}$ monocytes in peripheral sites and tissue-resident macrophages within the injured organ (Guilliams et al., 2018). During this initial injury, $Ly6C^{hi}$ monocytes enter tissue and differentiate into macrophages. Some monocytes will give rise to $Ly6C^{hi}$ macrophages, whereas others will downregulate Ly6C and join resident $Ly6C^{lo}$ macrophage populations that live within tissues (Epelman et al., 2014a). In the setting of organ transplantation, monocytes and macrophages are the dominant cell types that infiltrate an allograft during severe rejection episodes (van den Bosch et al., 2017). These $Ly6C^{hi}$ populations can contribute to alloimmunity through a variety of pathways, including antigen processing and presentation, costimulation, and pro-inflammatory cytokine production (van den Bosch et al., 2017).

In contrast, macrophages can also directly contribute to tissue repair. Resident cardiac macrophages are required for the remarkable regenerative capacity of neonatal myocardium (Epelman et al., 2014a). DC-$SIGN^+$ ($Ly6C^{lo}$) macrophages generate minimal inflammation and promote allograft tolerance through the secretion of interleukin 10 (Conde et al., 2015). However, cardiac macrophages are themselves highly heterogeneous in both cell surface marker expression and function. Resident $Ly6c^{lo}$ $CCR2^+$ macrophages are enriched in inflammatory pathways in resting conditions, and their depletion prior to syngeneic cardiac transplant limits early neutrophil recruitment and the initiation of the inflammatory response through DAMP signaling (Epelman et al., 2014b). After activation, it is not clear whether macrophages with different markers represent a spectrum of activation states or uniquely defined lineages. However, modulating early macrophage activation may shift the balance between different macrophage functions in a fashion that may promote allograft tolerance (Figure 1). In this issue of *Immunity*, Braza et al. (2018) identify a macrophage activation pathway that impairs cardiac allograft survival and demonstrate the efficacy of a myeloid-specific nanoimmunotherapy that increases long-term allograft survival by targeting the initial wave of peripherally recruited macrophages following transplantation (Braza et al., 2018).

The authors assessed the role of DAMP receptors dectin-1 and TLR4 in an allogeneic murine model of heart transplant. Graft-infiltrating macrophages expressed high levels of Ly6C, DECTIN-1, and TLR4, whereas DECTIN-1 (*Clec7a*)- and *Tlr4*-deficient mice exhibited reduced numbers of graft-infiltrating $Ly6C^{hi}$ macrophages together with increased numbers of $Ly6C^{lo}$ macrophages. In an elegant approach, Braza et al. (2018) proposed to target macrophages using high-density lipoprotein (HDL) nanoparticles with rapamycin (mTORi), which is a mammalian target of rapamycin (mTOR) inhibitor, encapsulated at the surface of the HDL. Such HDL nanoparticles have proven to preferentially target macrophages and work efficiently as carriers for lipophilic payloads such as rapamycin (Sanchez-Gaytan et al., 2015). Functionally, i.v. administration of mTORi-HDL markedly improved allograft survival with only three doses, which again correlated with reduced $Ly6C^{hi}$ macrophages and increased numbers of $Ly6C^{lo}$ macrophages in the allograft. These changes in macrophage composition were not observed after the administration of rapamycin alone, revealing the importance of the HDL-based targeting approach. Sorted macrophages from heart allografts had reduced tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6) expression and lactate production after *ex vivo* LPS stimulation from mice treated with mTORi-HDL. These data suggested

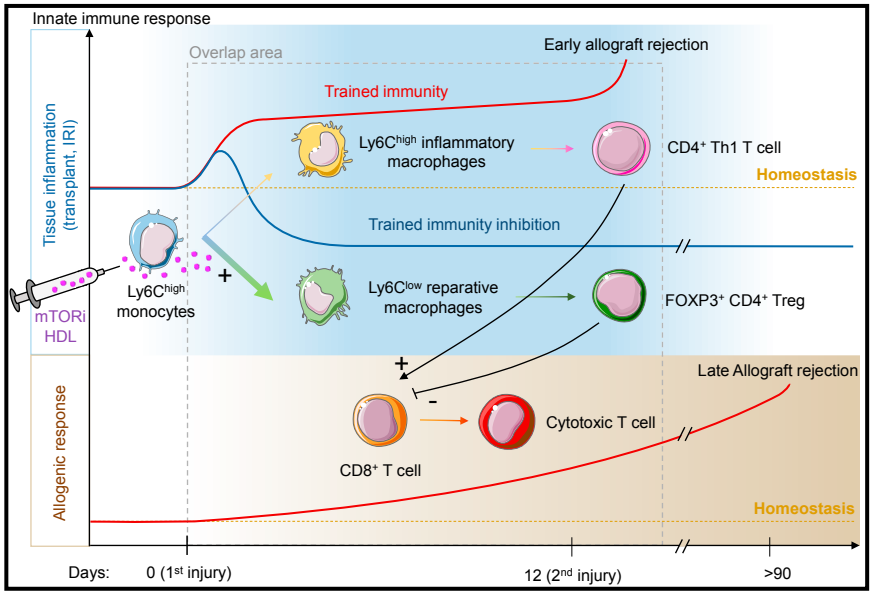

Figure 1. Inhibition of mTOR in Recruited Macrophages Improves Organ Survival after Transplantation

Schematic depicting the effects of mTORi-HDL therapy in the setting of cardiac allograft transplantation. The x axis (left to right) depicts time after transplantation. The upper blue panel depicts the macrophage response to the combination of surgical trauma and ischemia-reperfusion injury (IRI) after transplant. Increased blue shading refers to the intensity of the innate response, which occurs immediately after transplant. mTORi-HDL targets Ly6C^{hi} monocytes as they differentiate into Ly6c^{lo}CD169^{+} macrophages, which are required for the therapeutic effects of mTORi-HDL nanoparticles (increased graft survival). mTORi-HDL-conditioned, graft-associated cardiac macrophages have a blunted inflammatory response to *ex vivo* LPS stimulation due to the inhibition of early reprograming (innate training) from DAMP signals. mTORi-HDL-conditioned, graft-associated cardiac macrophages polarize CD4^{+} T cells into FOXP3^{+}CD4^{+} Treg cells and blunt CD8^{+} T cell activation. The lower beige panel refers to the secondary injury that occurs due to allogenic mismatch. Increased beige shading refers to the intensity of the adoptive immune response, which begins in the later stages of the innate immune response. In the absence of mTORi-HDL signaling, inflammatory (trained) macrophages further accelerate allograft rejection. Gray-dashed area (overlap area) indicates the time frame in which the studies were done.

that mTOR inhibition prevented the ability of cardiac macrophages to be further activated through TLR4 stimulation after *in vivo* treatment with mTORi-HDL (Braza et al., 2018). Importantly, *in vivo* mTORi-HDL-conditioned Ly6C^{lo} macrophages obtained from allografts inhibited CD8^{+} T cell proliferation while expanding immunosuppressive FOXP3^{+}CD4^{+} regulatory T (Treg) cells *in vitro* (Figure 1 schematic), suggesting that adaptive alloimmune responses may be indirectly blunted through a macrophage intermediate.

When assessing the role of recruited monocytes, it is often difficult to demarcate where the functions of monocytes end and macrophages begin. Braza et al. (2018) depleted differentiated CD169^{+} macrophages (not monocytes) in recipient mice, using the *Cd169*^{DTR} system, and show that recipient macrophages must be present for mTORi-HDL treatment to be effective. Remarkably, adoptive transfer of wild-type monocytes into mTORi-HDL-treated (CD169^{+} macrophage-depleted) mice restored allograft survival, presumably by delivering the cell type upon which mTORi-HDL therapy is targeting. Similarly, if Ly6C^{hi} monocytes were absent in mTORi-treated *Ccr2*$^{-/-}$ recipient mice mice, the therapeutic effect of therapy was again lost. Depletion of CD11c^{+} cells did not affect the therapeutic effects of mTORi-HDL, suggesting a pivotal role for the early, monocyte-derived macrophages in immune allograft survival, and not dendritic cells (DCs). Together these data suggest that after transplantation, recipient Ly6C^{hi} monocytes infiltrate the donor heart and begin differentiating into peripherally derived CD169^{+} cardiac macrophages, a subset required for the therapeutic effect of mTORi-HDL and subsequent graft tolerance. In addition, these data also suggest that monocyte-derived macrophages possess a tunable role in transplantation, where their presence is required to improve organ survival.

In organ transplantation, monocytes and macrophages must integrate multiple inflammatory signals that arise during a complex temporal cascade after the transplant. The response of a monocyte to any given stimuli is governed by whether that monocyte has been previously activated. Trained immunity refers to the ability of monocytes to exhibit memory characteristics, transmitted through epigenetic changes that occur after the first encounter with inflammatory stimuli (Guilliams et al., 2018). Use of mTORi has been reported to regulate monocyte and macrophage functions through trained immunity (Netea et al., 2016). Braza et al. (2018) demonstrated that β-glucan-stimulated mouse and human monocytes classically remodeled chromatin (H3k4 histone trimethylation), indicative of epigenetic changes associated with training, and that mTORi-HDL can inhibit the subsequent exaggerated inflammatory response to LPS (reduced TNF-α and IL-6 release and lactate production), indicative of mTOR-dependent training. Similar pro-inflammatory activity was detected in monocytes and macrophages infiltrating the allograft after transplantation, whereas *Clec7a*$^{-/-}$ mice, *Tlr4*$^{-/-}$ mice, and mTOR-HDL treatment blunted TNF-α, IL-6, and lactate production in graft-infiltrating macrophages after *in vitro* LPS challenge and inhibited H3k4 trimethylation of the respective promoters (Braza et al., 2018). Based on this evidence, it appears that graft-infiltrating monocytes encountered DAMPs that triggered mTOR-dependent epigenetic changes leading to increased pro-inflammatory cytokine potential—a pathway that increased allograft injury.

In transplantation studies, two injurious processes overlap in time, so it can be a challenge to determine where mTORi-HDL benefit precisely resides. Initially, acute and sub-acute inflammatory damage due to the combined effects of surgical trauma and IRI create an environment where surviving cardiac macrophages are epigenetically altered. Superimposed on the complexity of the innate immune response to tissue injury is the development of the allogeneic immune response (Figure 1). The initial and subsequent stimuli that influence monocytes and macrophages and epigenetically "train"

them are unclear. For example, is IRI producing epigenetic memory in macrophages resulting in an exaggerated response to the allogeneic transplant, or are there successive waves of allo-independent innate inflammation occurring during transplant that are amplified by training? Future studies, which can trigger mismatch (CRE-based systems) after healing has occurred, would be required to temporally separate both processes sufficiently so that one could inhibit only the transplant-induced innate training, and later, trigger mismatch in a truly sequential fashion.

As our understanding of monocyte and macrophage functional heterogeneity improves, it will be important to define whether peripherally derived macrophages are *en masse* targeted by mTORi-HDL. The authors show that macrophages are required for the beneficial effects of mTORi-HDL. It may be the case that recipient macrophages are required for a proper angiogenic or healing response to organ transplantation and if absent, there is simply too much injury for mTORi-HDL to be of benefit. Alternatively, after transplant, is mTORi-HDL targeting a subpopulation of myeloid cells within known heterogeneous monocyte and cardiac macrophage populations? For example, $Ym1^{+}$ $Ly6C^{hi}$ blood monocytes are a recently described blood monocyte subset that specifically promotes tissue repair, rather than inflammation (Ikeda et al., 2018). Does mTORi-HDL therapy affect this particular subset of $Ly6C^{hi}$ monocytes more so than other subsets? Or perhaps mTORi-HDL targets resident $CCR2^{+}$ cardiac macrophages, which acutely direct cardiac graft injury in mice and correlate with worsening cardiac function in human heart transplant (Bajpai et al., 2018)?

Although more work remains, the findings of Braza et al. (2018) are promising. Targeting the early, monocyte-dependent innate immune response represents a novel approach that has the potential to improve organ transplantation within not only the myocardium, but also other organs.

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