Hypothesis

Ex Vivo Tissue-Resident Macrophage Replacement Prior to Allogeneic Transplantation

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Ex Vivo Tissue-Resident Macrophage Replacement Prior to Allogeneic Transplantation

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Abstract: Organ shortage is one of the most pressing issues with regard to human mortality. This issue is further complicated by the fact that HLA matching is required for patients, leading to restricted choices for transplant. If organs or tissues from non- or partially-HLA–matched donors could be edited such they evade cytotoxic T cells as well as natural killer cells, it would ameliorate the organ shortage problem.

Keywords: normothermic machine perfusion; tissue-resident macrophage replacement; *E. coli* Nissle 1917; bactofection; CRISPR transposase; HLA-E

Introduction:

There are currently over 89,000 patients waiting for a kidney in the United States alone [1]. Patients who could benefit from partial liver lobe transplants may come second in terms of demand.

HLA matching is currently required for transplantation. Long-term immunosuppression is often necessary when there are HLA mismatches at least, leading to opportunistic infections. These infections are especially likely in HLA-mismatched donor organs - as a result of reduced cytotoxic T cell activity [2].

Thus, interventions that can reduce the immunogenicity of transplanted organs and prevent viral infection in those locales would be of great value.

Hypothesis:

Recent advances in normothermic machine perfusion afford time in which a given organ can be altered prior to transplant. In some cases this time window can be more than a week [3].

Given a multi-day window of time, the donors tissue-resident macrophages (TRMs) could be depleted using PLX3397, a small molecule CSF1R inhibitor, and at least partially repopulated by "off-the-shelf" TRMs [4–7].

These off-the-shelf macrophages could contain a small number of cytosolic *E. coli* Nissle 1917 (ECN) cells that are restrained in their replication via quorum sensing [8]. After TRM replacement with the edited cells is complete *ex vivo* or after transplantation of the organ, the TRMs could send non-replicating copies of the bacterial vector to surrounding parenchymal cells via secretory autophagy.

Bactofection could then ensue [9].

As one of the primary barriers to bactofection is the immunogenicity of the vector[9], and immunosuppression would be indicated after transplantation regardless, this would afford the vectors time to "bactofect" the parenchymal cells and enact HLA-E knock-in to the B2M locus[7]. This could prevent or at least reduce cytotoxic T cell and natural killer cell activity toward the donated organ.

Evaluation of the Hypothesis:

There may not be sufficient time for TRM replacement *ex vivo*. If not, the edited TRMs could secrete a CD64 or CD11b immunotoxin, to prevent host TRMs from interfering with the repopulation

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process [10]. As the edited TRMs replicate to repopulate the organ, the bacterial vectors would keep pace with their replication.

As the off-the-shelf TRMs would not have functional MHC Class I complexes, this could effectively shield the bacteria from the immune system.

The bacteria would also be made dependent on a peptide from the off-the-shelf TRMs for continued survival.

IR8$^{-/-}$ TRMs could be employed here to prevent xenophagy of the bacterial vectors [11]. This might further reduce immunogenicity by lowering the levels of PAMPs or DAMPs produced through xenophagy and vector replication back to quorum sensing levels.

Bacterial competence circuitry induced by the periodic expression of a peptide by the TRMs would allow a small percentage of the already limited bacterial population to lose their replication potential and be donated via secretory autophagy to the extracellular space [12–16]. These bacteria would no longer be dependent on the TRM peptide for survival, however. Flagellar motility would then allow them to reach cells throughout the organ [17].

Expression of an adhesin that binds the target cell type, the *Yersinia pseudotuberculosis* invasin, and listeriolysin O would allow the ECN vectors to enter parenchymal cells [18,19].

After egress, a noisy Deadman switch could be utilized based on the *Listeria monocytogenes* ActA promoter [20–22]. Thus, the donated bacterial vectors would lyse after a certain amount of time. This could reduce immunogenicity by decreasing the number of extracellular bacteria that may have strayed outside the transplanted organ.

Once inside a target cell, the bacteria would utilize the *actA* promoter to express a phage lysin [23]. They would lyse to release linear, double-stranded DNA and nuclear localization sequence-containing proteins that bind to said DNA [24,25]. They would also release proteins that allow for the knock-in of HLA-E at the B2M locus, to disrupt MHC Class I expression and preclude natural killer cell cytotoxicity[7].

CRISPR transposases may be appropriate here [26].

One other edit that the bactofection method could make to the donor cells is to install "Double-stranded RNA (dsRNA) Activated Caspase Oligomerizer" (DRACO) in a safe harbor locus - perhaps under an inducible promoter [27]. This could help to prevent a multitude of viral infections in the donor organ.

Genes encoding antimicrobial proteins or peptides could also theoretically be installed in the donor cells [28].

It may be advisable or at least non-problematic to kill the bacteria but leave the off-the-shelf TRMs in the donor organ.

If not, a tetracycline-inducible caspase 9 based on a non-immunogenic RNA system could be employed for lysis of the TRMs after treatment [29]. The bacteria could be killed with this molecule as well via an inducible system or the effects of the antibiotic itself, although they would soon die after TRM lysis.

Alternatively, as the TRMs would already lack MHC Class I complexes, caspase 9 could be induced by rapamycin or a rapalog-dimerizable transcription factor instead [30].

Consequences of the Hypothesis and Discussion:

Countless patients around the world require transplants. The kidney may be the organ that is most needed. Interventions that can negate or reduce the need for HLA matching would be of great use.

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