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Editorial

Advancing the technology for head transplants: From immunology to peripheral nerve fusion

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In this editorial, we hash out the details of two major aspects of the technology behind head transplants (HEAVEN), one having to do with immunosuppression (IS), the other on the reconnection of peripheral nerves at neck level.

IMMUNOTOLERANCE

In the context of HEAVEN,[5] the donor body would reject the head (although it cannot be excluded that resident head immune cells in the head can mount a reaction against the body), with rejection of tissues not protected by the blood–brain barrier (i.e., the brain) such as the pituitary gland or the spinal cord (and of course the face skin and muscles) especially critical. This necessitates IS with a protocol similar to facial and limb transplants. Sirolimus, with its minimal neurotoxicity and pro-neuroregenerative properties (despite its interference with wound healing), is especially indicated. However, long-term administration of these medications results – as is well known – in significant morbidity and mortality, including nephrotoxicity, infections, neoplasm, and cardiovascular diseases. Equally important, chronic rejection (graft-versus-host-disease [GVHD]) is not prevented, even with maximal IS. Therefore, induction of allograft tolerance – so that no drug is required – is a sine-qua-non, as previously indicated.[4]

In the 21st century, tolerance induction through chimerism (full or partial) is a clinical reality. Tolerance to HLA matched and mismatched living donor kidney transplants with complete withdrawal of IS drugs without subsequent rejection for up to 14 years of observation has been achieved in more than 50 patients enrolled in trials in four medical centers after the establishment of transient or persistent chimerism (with nonmyeloablative conditioning regimens employing thymoglobulin, belatacept, and bone marrow transplantation plus rituximab). Complete drug withdrawal without chimerism was reported in a prospective trial of liver transplantation combined with injection of regulatory T cells. IS drug minimization without rejection was reported in recipients of living donor kidney transplants after injection of recipient regulatory T cells or injection of donor regulatory monocytes or dendritic cells.[10] In one HEAVEN scenario,[14] the donor's bone marrow would be populated with the bone marrow cells of the recipient after the donor is sublethally irradiated (with vital organs shielding), including the thymus, and its periphery is depleted of donor T cells (with Thymoglobulin), B cells (with Rituximab), plasma cells (with Carfilzomib), and macrophages (with Alemtuzumab). Thereafter, the body is immunologically reconstituted with the recipient's bone marrow and

peripheral stem cells. The entire process should take no more than 1 month or less (alternatively, the donor's immature dendritic cells [imDCs] are isolated and primed *in vitro* with Class I and Class II immunodominant transplantation allopeptides of the recipient (head) and re-injected into the immune-depleted donor body for thymus education). As one can see, this is a labor-intensive regimen, which is still fraught with possible complications (including delayed radiation toxicity).

Apoptotic cell-based therapies represent a novel option that may improve graft survival and also be effective for the treatment of GVHD.[12,15] In particular, vaccination with apoptotic donor leukocytes (ADLs) represents a non-chimeric strategy for inducing donor antigen-specific tolerance in transplantation. Leukocytes treated *ex vivo* with the chemical cross-linker ethylcarbodiimide (ECDI) undergo rapid apoptosis after intravenous (IV) infusion.[11] Hering's group[17] showed that two peritransplant infusions of ADLs under short-term immunotherapy given 1 week before transplantation (Antagonistic anti-CD40 mAb 2C10R4 IV at 50 mg/kg on days −8, −1, 7, and 14; Rapamycin PO from day 7 to day 21 posttransplant; concomitant anti-inflammatory therapy: (i) tocilizumab at 0 mg/kg IV on days −7, 0, 7, 14, and 21, and (ii) etanercept at 1mg/kg IV on days −7 and 0 and 0.5 mg/kg subcutaneous on days 3, 7, 10, 14, and 21; last day of IS: day +7) induced long-term (≥1 year) tolerance to islet allografts in 5 of 5 macaques. Unlike the mixed chimerism strategy, this regimen effectively induced stable tolerance without requiring irradiation, indiscriminate generalized T cell deletion, simultaneous hematopoietic stem cell transplantation, or a course of either calcineurin inhibitors or anti-CD8-depleting antibodies for control of early posttransplant direct pathway activation and associated toxicities;[19] unlike other antigen-specific strategies involving soluble peptide and altered peptide ligand therapy, ECDI-fixed leukocyte infusions are not associated with the risk of anaphylaxis or other safety concerns.[1] Finally, in contrast to other cell-based tolerance strategies under evaluation;[16] this regimen does not require the adoptive transfer of regulatory cells. These authors concluded that their "study suggests that the long-pursued goal of transplantation tolerance is attainable with a non-chimeric ADL strategy that establishes a sustained and antigen-specific regulatory network." This is a clear option for HEAVEN.

PERIPHERAL NERVE FUSION

As illustrated in a previous paper,[13] during a head transplant, both the phrenic nerves and the recurrent laryngeal nerves are spared. However, the vagi are not and must be transected and repaired. Simple micro-suturing of a transected nerve may be ineffective, and behavioral recovery takes months to manifest. As mentioned,[5] the Bittner's protocol of polyethylene glycol (PEG)-induced nerve fusion looks promising in this setting, in light of the excellent outcomes and rapidity of recovery.[3] However, recent studies have not been positive, and Bittner pushed back against the methodology and conclusions of these authors.[2] In our recent study, we applied PEG 600 (0.5 mL) in a model of sciatic nerve transection without following the more convoluted Bittner's protocol and found that PEG is effective, especially if injected inside an arterial chamber inside of which the two severed stumps are inserted.[18]

The question arises whether other fusion protocols exist that might complement the PEG approach. In the 20th century, Luis De Medinaceli contributed one such approach, which is of particular interest (unfortunately he died in a car crash in 1996). He started observing how errors in the direction of peripheral nerve regenerating sprouts (mismatching) lead to suboptimal results and failures. He also noted how even guillotine-like devices used to make "clean" nerve sections cannot eliminate the crushing, twisting, and tearing injury that is inflicted by even the sharpest blade (lasers do not, but coagulate the tissue and are thus of no use in this context). He thus froze the stumps, resulting in smooth surfaces of transection with minimal crush injury and avoided the cell damage stemming from freezing-thawing (due to intracellular icicle formation) by bathing the nerve in appropriate fluids, carefully avoiding supercooling and keeping duration and depth of cooling to a minimum (e.g., −180°C for 30 s or −5°C for 5 min are both harmful to the nerve cells and axons). The two fluids he developed included chlorpromazine (1 mM) and polyvinyl alcohol (PVA) 15% w/v (plus NaCl 140 mM and KCl 4 mM) for first stage protection (fluid 1) and chlorpromazine and PVA as per fluid 1 plus NaCl (10 mM), KH_2PO_4 (120 mM), NaCO3H (5 mM), imidazole (40 mM), and potassium hydroxide to adjust pH to 6.8 (fluid 2).[6] Still, even two neatly severed stumps would be damaged by microsutures. He thus patented an anti-retraction device based on the principle of Saint Venant (1856), which deals with the distribution of tensile forces in elastic bodies. In particular, sutures placed at the repair site contribute to the disorganization of the nerve structure, whereas anchoring sutures placed at an appropriate distance from the stumps (equal to at least one and a half times the diameter of the structure) eliminate stress at the site of repair. The result is excellent histological appearance, very precise stump approximation, no interposed foreign material and very little disruption in fiber direction; functional results have been favorable.[8] In the only clinical study available,[7] the two stumps of a traumatized nerve were treated with 1–2 drops of 2 mM solution of chlorpromazine HCl in saline, freed, and then sutured to a support made of a bioabsorbable material in such a way that they slightly overlapped. Fluid 2 was then applied, and the ends of the stumps briefly frozen (−2/−7°C; 30–150"). The solidified stumps were trimmed

with a sharp blade and immediately thawed. Additional stability came from adding a drop of fibrin glue (PEG works equally well).[9] In sum, his idea was thus to minimize the secondary damage that accompanies peripheral nerve transection: chlorpromazine would thwart the deleterious effects of calcium influx into the axonal compartment and PVA would stall stump swelling due to water influx. This would be especially vital in the case of the distal stump that would undergo fragmentation and degeneration. In conclusion, the De Medinaceli approach, in part or whole, could find a place in the HEAVEN setting and could be interleaved with PEG, should appropriate experiments confirm its usefulness.

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Conflicts of interest

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