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Induced pluripotent stem cell therapy in transplantation—A potential role for immunologic tolerance

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Abstract

Induced pluripotent stem cells (iPSCs) hold the potential for future development of genetically identical tissues from almost any mature cell lineage. For clinical applications in cell therapy and transplantation, it may provide a means to one-day restore dysfunctional or damaged tissue without the need for immunosuppression. A recent study by de Almeida et al. published in the journal *Nature Communications* indicates that iPSCs may indeed elicit an immune response that evolves as cells differentiate toward maturity to induce a state of tolerance within a recipient animal. If these early findings hold true, it suggests a possible explanation for self-recognition of mature cells derived from iPSCs for use in future therapeutic interventions in transplantation such as cellular therapy or tissue engineering.

Induced pluripotent stem cells (iPSCs) have been touted as a ‘game changer’ for modern medicine due to the ability to differentiate into any cell type and mimic its phenotype, either normal or deficient (1). This relatively new technology provides a platform to sample a mature cell from a patient to model genetically-based diseases *ex vivo* or generate cells for new therapeutic applications, which the donor’s immune system would recognize as ‘self,’ allowing for autologous transplantation without immunosuppression. Almost any mature cell type, though typically fibroblasts, may be reprogrammed to pluripotency to form iPSCs by introducing Yamanaka Factors (Oct4, Sox2, cMyc, and Klf4) via infection with genetically integrative retroviruses or, more recently, through transfection of episomal

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vectors or other non-DNA integrative techniques. The result is an artificially created stem cell, derived from adult tissue, with the capacity for self-replication and directed differentiation into all three germ layers depending upon maturing conditions. iPSCs may be directed toward defined paths of differentiation by addition of specific growth factors and small molecular agonists/antagonists of key regulatory elements providing for *in vitro* differentiation or may undergo maturation *in vivo* after insertion into early-stage embryos that grow to form chimeric animal models.

The future implication of this technology to clinical transplantation is the potential of iPSCs, and their mature progeny, to restore damaged tissue and avoid an alloreactive immune response, to be recognized as 'self' by the immune system of the original cell donor. Though this may not be as important for drug discovery and toxicity evaluation *ex vivo*, the ability of iPSCs to evade immune recognition is intrinsic to its capacity to offer a 'personalized medicine' approach to cell therapy, transplantation and even tissue engineering while obviating the need for immunosuppression. The clinical implications of this approach was realized in September when a 70-year old Japanese patient became the first recipient to be treated with autologous iPSC-derived retinal pigmented epithelium after her own dermal cells were reprogrammed to replace damaged retinal tissue from age-related macular degeneration (2).

iPSCs are artificially generated and so the assumption that these cells express a repertoire of only 'self' antigens remains unsubstantiated and, indeed, there has been inconsistent findings characterizing the immunogenicity of iPSCs over the past several years. Zhao et al. surprisingly revealed in *Nature* in 2011 that iPSCs derived from B6 mouse embryonic fibroblasts, when injected subcutaneously into syngeneic B6 recipients, led to T-cell infiltration, tissue necrosis, and failure to produce teratomas, providing the first indication that undifferentiated iPSCs may be immunogenic in syngeneic hosts (3). Teratoma formation in rodents is the classical *in vivo* proof of 'stemness' (e.g. pluripotency) for both iPSCs and embryonic stem cells (ESCs) and is used to assess if cells can evade immune surveillance to persist, and differentiate into all three germ cell lineages, within a recipient animal. However, the direct implication of this assay to the clinical use of iPSC and ESCs is questionable as their mature progeny, not the parental pluripotent cells, will be used to treat clinical conditions in patients such those in the age-related macular degeneration study in Japan.

One notable paper by de Almeida et al. published in the journal *Nature Communications* supports the observation by Zhao and evaluated the immune response of murine autologous iPSCs and noted that these immature cells are also targeted and destroyed by the immune system. However, de Almeida et al. then went further to assess the immunogenicity of the endothelial cell (EC) progeny differentiated through an *in vitro* strategy (4). Consistent with the Zhao report, the authors of this recent study show that iPSCs implanted into hind limb of immunocompetent FVB mice were eradicated by 21 to 42 days depending upon the mechanism of iPSC reprogramming (integrative vs. non-integrative strategy, respectively). Conversely, implantation of iPSCs into immunodeficient NOD/SCID/IL-2 receptor γ chain knockout mice permitted teratoma development as did implantation into IFN- $\gamma^{-/-}$ recipient mice, while priming of wild-type recipient rodents with iPSCs led to accelerated immune-

mediated rejection. Taken together, the de Almeida study lends further support to the notion that syngeneic iPSCs elicit a robust T-cell immune response, though the exact mechanism is unclear.

Nonetheless, the clinically important question relates to the identity of the target cell population that holds the most clinical relevance—the mature cellular progeny. This population is of major interest for transplantation and cellular engineering, and conceivably the antigen repertoire may change during maturation, thus affecting its immunogenicity. To address this, de Almeida et al. differentiated parental iPSCs into ECs *ex vivo* to obtain a pure population after sorting for CD144 and CD 31. Mature, iPSC-derived ECs were implanted into the hind limb of syngeneic mice with limb ischemia. These recipient animals exhibited improved blood flow by laser Doppler imaging with some muscle atrophy but without signs of teratoma formation. Both parental iPSCs and iPSC-derived ECs were followed longitudinally during the course of the study by *in vivo* expression of luciferase. Unlike the parental iPSC population, which was eradicated within the first 42 days, differentiated ECs persisted throughout the 62-day study period and could be followed serially through bioluminescence. T-cell infiltration was found in iPSC grafts as well as those containing either iPSC-derived ECs or syngeneic aortic endothelial cells (AECs). AECs were used as a control for cells recognized immunologically as ‘self’. The inflammatory response directed at parental iPSCs was more robust with increased expression of granzyme-B, IFN- γ , and perforin while iPSC-derived ECs expressed higher levels of IL-10 and TGF- β , both potent mediators of tolerance. The central role of IL-10 in this system was illustrated by accelerated clearance of iPSC-derived ECs in recipient mice treated with anti-IL-10 antibodies. The role of CD4⁺ T-cells expressing FoxP3 responding to parental iPSCs and differentiated ECs is less clear, however, as comparable levels were found in grafts of iPSCs or iPSC-derived ECs, yet CD4⁺ T-cells expressing FoxP3 were higher in both these grafts than in those derived from ‘self’ AECs. Transcription of FoxP3 had a higher trend in both mature AECs and iPSC-derived ECs, compared to parental iPSCs, without reaching significance, together suggesting that other regulatory T-cell populations may be in play.

Using single-cell PCR, de Almeida et al. went further to assess gene expression in T-cells and suppressor macrophages responding to grafts containing iPSC, iPSC-derived ECs or ‘self’ AECs to find gene signatures consistent with the suppressor function of Tregs and T-cell unresponsiveness, including IL-10, TGF- β , and CTLA4, in mature iPSC-derived EC and AEC grafts. By employing high-throughput sequencing of the T-cell receptor (TCR) rearrangement through an assessment of CDR3 length, de Almeida found that T-cells infiltrating iPSC grafts were oligoclonal, with the top five clones making up 20–55% of total TCR diversity while T-cells found in iPSC-derived ECs or ‘self’ AECs made up 15–20% or 10–25% of overall TCR diversity, respectively. Moreover, using a statistical measurement of sample overlap (Bhattacharyya coefficient), the authors show that T-cell clones which infiltrate iPSC grafts are statistically distinct from T-cells attracted to either iPSC-derived ECs or AECs, while T-cells found within grafts of both mature endothelial cells (iPSC-derived ECs or AECs) are indistinguishable from each other, together suggesting that the immune response to iPSCs are distinct from their mature progeny.

The important lessons of de Almeida et al. point to a model in which pluripotent cells, or their early progenitors, elicit a T-cell mediated immune reaction that changes in response depending upon the differentiation state of the iPSC-derived cell. The immunological driver of this response is unknown, but may be due to *in vitro* culture conditions and/or slight variations in reprogramming strategies that may vary depending upon the animal model, limiting the ability to correlate these findings to what may be observed in clinical studies. Alternatively, the location of iPSC implantation may play a role in graft maintenance. Guha et al. transplanted iPSCs or their mature progeny (ECs, neurons or hepatocytes) under the renal capsule of syngeneic rodents and found that pluripotent stem cells formed teratomas and none of the cells elicited an immune response that was robust enough to induce rejection, though some T-cells were found in grafts containing pluripotent cells (5). Araki et al. found that both iPSCs produced by non-integrative methods and ESCs led to teratoma formation after 4 to 5 weeks post subcutaneous injection into flanks of syngeneic recipients without marked T-cell infiltration. Both the Guha and Araki studies report disparate immunological findings with regard to the immature iPSC population compared to de Almeida and Zhao, and so further studies are needed to definitively clarify the mechanisms leading to autoreactivity toward parental iPSCs. For differentiated cells, the Araki study incorporated iPSC clones into an embryo aggregation strategy to produce highly chimeric mice with mature iPSC-derived dermal and bone marrow tissue using an *in vivo* differentiation method. Like the de Almeida study, transplantation of iPSC-derived mature tissues (bone marrow and dermal tissue in the Araki study) resulted in graft acceptance with little inflammation.

The emergence of an autoreactive immune response to iPSCs or their mature progeny may not necessarily detract from the therapeutic benefit of iPSC technology, but would indicate a need for a degree of immunosuppression. Coupled with this possibility and the understanding that derivation and standardization of specific, patient derived stem cells for personalized therapy would require significant labor, finances and time, an alternative approach currently being investigated in several countries is the development of standardized stem cell banks of iPSCs with genetically-defined HLA haplotypes (6, 7). Recently, large animal models have been developed to investigate iPSCs in a more clinically relevant setting in preparation for human clinical studies. In pigs, swine leukocyte antigen-matched iPSCs elicit an attenuated innate immune response (8). Though such an approach using HLA-matched cells would require immunosuppression for human therapy, it would provide defined allogeneic cell populations developed under standard reprogramming strategies that may better restrict or control expression of genetic or epigenetic factors that trigger immune recognition. In pre-clinical studies using cynomolgus monkeys without immunosuppression in support of the Japanese age-related macular degeneration trial, cell sheets of iPSC-derived retina pigmented epithelium were not rejected after one year in the autologous recipient whereas allogeneic monkeys developed fibrous tissue within the eye with leakage of fluorescein, indicating rejection, together suggesting that mature iPSC progeny should be accepted in autologous human recipients without the need for immunosuppression (9).

Taken as a whole, the small amount of data so far preliminarily suggests that variation between immature iPSC clones (and possibly their differentiated progeny) likely elicit a degree of inflammation when implanted into syngeneic recipient animals, though are not always rejected, possibly dependent upon the magnitude and nature of the immune response. iPSCs have the capacity to be rejected in syngeneic recipients, as noted in some studies, yet the causative mechanisms that modulate both the innate and adaptive immune response remain elusive though may be due to genetic or epigenetic changes that occur during the reprogramming process or may be related to the age and origin of the initial somatic cell that is driven to pluripotency. CD8+ cells were predominantly found in iPSC grafts of the de Almeida study, potentially suggesting a virus-type response that may relate to the method of cellular reprogramming. Depletion of CD4+ T-cells may provide further insight into whether rejection is dependent upon conventional T-cell help. The encouraging news for iPSCs in cell therapy is that differentiated cells appear to be accepted in rodents through a signature suggestive of peripheral tolerance; however, a similar in-depth investigation has not been conducted in primates. Nonetheless, with new large animal models, such as those used in support of Japan's age-related macular degeneration study, clinically translatable findings delineating immune mechanisms may be on the horizon. The ability to monitor persistence of specific T-cell clones by high throughput screening allows for the ability to track the durability of the response over time as further studies are needed to completely address the issue of immune regulation using iPSCs, particularly because of the apparent dynamic immunogenicity of iPSCs as cells mature. One form of cellular therapy, which may have the highest impact on modern medicine, is the ability to correct genetic defects through a process termed genome editing in which a missing gene is inserted into the DNA of iPSCs to rectify a genetic defect. It remains to be seen if these newly expressed proteins would function as neo-antigens for T-cells, which would not have been deleted by negative selection in the thymus. Nonetheless, as iPSC technology is a relatively new and growing field, its specific application to clinical transplantation and its role in personalized medicine will be further clarified by a refined understanding of cellular mechanisms that influence immunogenicity and tolerance to iPSCs and their progeny.

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Abbreviations

iPSC	induced pluripotent stem cell
ESC	embryonic stem cell
EC	endothelial cell
ASC	aortic endothelial cell
TCR	T-cell receptor

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