



## Commentary

## Human ES cell-derived dendritic cells: Meeting the challenge of immune rejection in allogeneic cell therapy

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Stem cells provide attractive opportunities for cell-based therapies because they combine two unique properties in one cell: a high self-renewal activity and a broad differentiation potential, which puts stem cells aside from most other somatic cells. In this context pluripotent stem cells, such as embryonic stem cells (ES cells) or induced pluripotent stem cells (iPS cells), are particularly appealing, since they are essentially immortal and can develop into all cell types of our body and thus employed for cell-based therapies [1].

Patient-specific iPS cells are obtained by the reprogramming of somatic cells and conceptually provide an unlimited cell source of autologous cells without the risk of immune rejection. However, the potential immunogenicity of iPS cell-derived cell products has been subject of debate over the past years [1,2]. Additionally, autologous iPS cells tailored to a specific patient come along with high costs, long production time, risk of genetic instability and elaborate quality management.

Our current knowledge of human ES cells and their differentiated progeny is more advance than it is for human iPS cells. Therefore, several clinical trials of human ES cell-derived cell products are ongoing, which address an urged medical need for devastating diseases, such as Parkinson's disease or macular degeneration (ClinicalTrials.gov). Human ES cell-derived cells are allogeneic and thus their transplantation entails the risk of immune rejection by the recipient.

To avoid immune rejection, immunosuppressive therapy is required, which puts an additional burden on patients and can be associated with high toxicity and severe side effects, such as risk of infections. Thus over the past several years many avenues have been tested for immune protection of ES cell-derived allografts, such as expression of immune suppressive molecules or disrupted expression of major histocompatibility complex (MHC) class I and class II

molecules [3]. The generation of tolerogenic dendritic cells from pluripotent cells [4,5] might represent yet another avenue for overcoming immune rejection.

Dendritic cells (DCs) are professional antigen-presenting cells of the immune system with a pivotal function in immunity and immune tolerance [6]. DCs develop from hematopoietic stem cells in bone marrow and are classified into three main subsets, classical DC type 1 and 2 (cDC1 and cDC2, respectively) and plasmacytoid DC (pDC), based on surface marker expression, function and ontogeny. Further to this, DC function is context-dependent: activated DCs induce immunity and non-activated immature DCs promote or maintain tolerance, and both properties are being explored in medical therapy [6]. For example, monocyte-derived DCs are activated by inflammatory stimuli and employed for tumor vaccines. Conversely, monocyte-derived DCs are treated with agents to maintain an immature tolerogenic phenotype. In addition, iPS cell-derived DCs were reported to display tolerogenic properties [4,5].

In a recent study in *EBioMedicine* Todorova et al. [7] derived DC-like cells (DCLs) from human ES cells expressing CTLA4-Ig and PD-L1, which induce immune tolerance specific for DCL-specific HLAs. Cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed death-ligand 1 (PD-L1) are critical for maintaining peripheral tolerance. CTLA4-immunoglobulin fusion protein CTLA4-Ig disrupts the T cell costimulatory pathways and PD-L1 activates T cell inhibitory pathways. CTLA4-Ig and PD-L1 were knocked into the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus in human ES cells [8] and these ES cells were differentiated into DCLs, referred to as CP DCLs.

Such CP DCLs showed low expression of co-stimulatory molecules, MHC class II, and the DC migration guidance receptor CCR7, and this low expression was maintained even after robust inflammatory stimuli. CP DCLs were impaired in T cell activation function and promoted the generation of regulatory T cells (Treg cells). CP DCLs protected parental ES cells from immune rejection in an adoptive transfer model of humanized mice. CP DCLs also protected ES cell-derived cardiomyocytes and smooth muscle cells from immune rejection. This all occurred without inducing systemic immune suppression and the induced immune tolerance was specific for the parental ES cells and their progeny.

The paper adds to the array of approaches to prevent allogeneic immune rejection of ES cell-derived allografts. However, one

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of the attractive features of the strategy by Todorova et al. is preventing immune rejection without the need for persistent systemic immune suppression. DCs and also CP DCLs are short-lived cells and dampened immune rejection was monitored for up to 4 weeks. Thus, whether immune tolerance and graft surveillance induced by CP DCLs is long-term and sustained still needs to be determined.

Pluripotent stem cells as source of DCs allows employing a rich tool box for genetic modification, such as knocking in genes shown by Todorova et al. and knocking out genes with CRISPR/Cas genome editing [9], which is particularly efficient for pluripotent cells. However, the generation of DCs from pluripotent stem cells, including ES cells and iPS cells, is difficult and consequently Todorova et al. refer to their ES cell-derived cells as DC-like cells (DCL). ES cell differentiation into DCs recapitulates early steps of human development and frequently yields embryonic cell derivatives. DCs in the adult are derived from bone marrow and this calls for further refinement of protocols for ES cell and iPS cell differentiation into DCs, including specific DC subsets, that fully recapitulate bone marrow-born DCs and their functions.

### Contributors

The author confirms sole responsibility for the conception and preparation of this invited Commentary.

### Declaration of Competing Interests

MZ declares no conflicts of interest.

### References

- [1] Yamanaka S. Pluripotent stem cell-based cell therapy - promise and challenges. *Cell Stem Cell* 2020;27(4):523–31.
- [2] Boyd AS, Rodrigues NP, Lui KO, Fu X, Xu Y. Concise review: immune recognition of induced pluripotent stem cells. *Stem Cells* 2012;30(5):797–803.
- [3] Xu H, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, et al. Targeted disruption of HLA genes via CRISPR-Cas9 generates iPSCs with enhanced immune compatibility. *Cell Stem Cell* 2019;24(4) 566–78 e7.
- [4] Cai S, Hou J, Fujino M, Zhang Q, Ichimaru N, Takahara S, et al. iPSC-derived regulatory dendritic cells inhibit allograft rejection by generating alloantigen-specific regulatory T cells. *Stem Cell Reports* 2017;8(5):1174–89.
- [5] Sachamitr P, Leishman AJ, Davies TJ, Fairchild PJ. Directed differentiation of human induced pluripotent stem cells into dendritic cells displaying tolerogenic properties and resembling the CD141(+) subset. *Front Immunol* 2017; 8:1935.
- [6] Mildner A, Jung S. Development and function of dendritic cell subsets. *Immunity* 2014;40(5):642–56.
- [7] Todorova D, Zhang Y, Chen Q, Liu J, He J, Fu X, Xu Y. hESC-derived immune suppressive dendritic cells induce immune tolerance of parental hESC-derived allografts. *EBioMedicine* 2020.
- [8] Rong Z, Wang M, Hu Z, Stradner M, Zhu S, Kong H, et al. An effective approach to prevent immune rejection of human ESC-derived allografts. *Cell Stem Cell* 2014;14 (1):121–30.
- [9] Sontag S, Forster M, Qin J, Wanek P, Mitzka S, Schüler HM, et al. Modelling IRF8 deficient human hematopoiesis and dendritic cell development with engineered iPSCs. *Stem Cells* 2017;35(4):898–908.