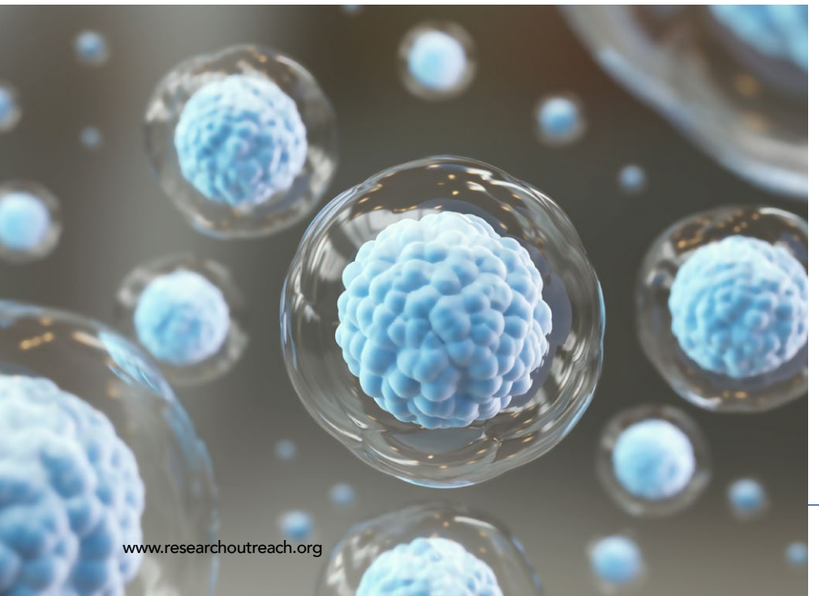
A new generation of induced pluripotent stem cells for regenerative medicine

Embryonic stem cells or induced pluripotent stem cells are unlimited sources for the generation of cell derivatives for the regeneration of failing organs. However, such cell transplants are subject to immune rejection by the recipient's immune system. Drs Sonja Schrepfer and Tobias Deuse from the University of California, San Francisco, have developed a new generation of immune-edited induced pluripotent stem cells that could constitute a source of universally compatible cell products.

Stem cells have the unique ability to be culture expanded and differentiated into various tissue-specific cell types. They hold great potential for treating a broad range of diseases in which crucial cells have died or vanished during the disease process. Regenerative cell therapy aims to transplant or inject healthy cell products to replace or regenerate the damaged or lost cells or tissues. Prime candidates for regenerative cell therapy include heart failure, for which cardiomyocyte transplantation is being tested, and type 1 diabetes mellitus, for which pancreatic β cells and islets are being developed, among others.

Immunogenicity is the ability of a foreign cell or protein, which functions as an antigen, to provoke an immune response, and this constitutes one of the main challenges in cell transplantation. An immune response leads to immune rejection and the transplanted cells are eliminated. In order for transplanted cells to survive long term, they must go undetected by the patient's immune system or not be recognized as foreign.



In their early work, Dr Schrepfer and Dr Deuse showed that embryonic stem cells, which were initially thought to be a very promising source of less immunogenic cells for therapy, could induce an immune response resulting in rejection.

THE LIMITS OF EMBRYONIC STEM CELLS

Embryonic stem cells (ESCs) are derived from embryos at an early developmental stage, before the time that implantation would normally occur in the uterus. ESCs can differentiate into multiple adult cell types *in vitro*, such as cardiac cells or neurons.

Major histocompatibility complex (MHC) molecules are cell surface proteins that play a key role in immune recognition. These proteins are highly polymorphic and present endogenous peptides to immune cells. If immune cells recognise foreign antigens, an immune response against the cells is induced and the cells undergo cytotoxic killing. ESCs have been shown to express low levels of MHC antigens, which makes them more likely to evade the immune system.

However, Dr Schrepfer's group showed that the low levels of immunogenicity-related molecules expressed on ESCs were still sufficient to trigger an immune response and result in rejection[1]. Survival of transplanted ESCs, however, was achieved in immunodeficient mice lacking a functional immune system[2]. This seminal early work drew the attention of the stem cell community and sparked much of the subsequent immunology research related to stem cells and regenerative therapy.

MINOR HISTOCOMPATIBILITY ANTIGENS

Dr Schrepfer and Dr Deuse revealed that minor histocompatibility antigens (mHAs) can emerge as transplant-relevant antigens in the context of cell transplantation. Some mHAs can be recognized by the recipient's immune system and induce an immune response even without MHC mismatches.

The team described the immunogenicity of H-Y mHAs, antigens that are encoded by the Y chromosome and therefore present only in males. These antigens can cause immune responses in female recipients. Transplants of cells derived from male mice survived long-term in male recipients but were rejected in female recipients of the same inbred strain[3]. Thus, the gender of the donors for cell products should be carefully considered.

The early work of Dr Schrepfer and Dr Deuse drew the attention of the stem cell community.

To categorically avoid problems with immune rejection, many companies develop manufacturing capabilities for the generation of autologous cell products. One way of generating patient-specific ESCs is via the technique of somatic cell nucleus transfer (SCNT), which transfers the nucleus of a somatic (body) cell from the patient into an enucleated oocyte (an egg that has had its own nucleus removed) from a donor. After several days in which the oocyte divides, ESCs with the patient's nuclear DNA can be isolated and expanded. However, these ESCs contain mitochondria, small organelles that possess their own DNA, which are inherited from the oocyte donor. The immunological relevance of this phenomenon was unknown until Dr Schrepfer and Dr Deuse discovered transplant-relevant mHAs in mitochondrial DNA (mtDNA)[4]. Despite the ability to generate ESCs and various tissue-specific cell types possessing the patient-specific DNA, such cell products were subject to immune rejection if their mtDNA showed mismatches. The SCNT technology was shown not to circumvent the immune hurdle for cell transplantation.

AUTOLOGOUS INDUCED PLURIPOTENT STEM CELLS

Autologous induced pluripotent stem cells (iPSCs) are generated from a patient's somatic cell via a process called reprogramming. Somatic cells are treated with factors or agents to erase their tissue-specific epigenetic marks, such as DNA methylation, and remodel them into an undifferentiated, pluripotent phenotype. Such patient-specific iPSCs are fully MHC and mHA identical to the somatic cell donor. Derivatives from autologous iPSCs should thus not express foreign antigens and should avoid immune rejection in this specific patient. At least that has been the widespread belief in the field.

Stem cell-derived products can induce an immune response, which may limit the success of regenerative medicine approaches.

Dr Schrepfer and Dr Deuse carefully investigated how iPSC immunogenicity can be affected by reprogramming, in vitro cell expansion, and cell differentiation. The unexpected finding was that reprogramming and large-scale cell expansion in vitro allows for the emergence of mtDNA-encoded neoantigens: these are antigens that developed during the process and were not present in the somatic starter cell[5]. Mechanisms for the emergence of neoantigens include mutations and heteroplasmic variant drift, common mtDNA phenomena in all rapidly-proliferating tissues. Mutation rates during reprogramming have been reported to be up to nine-fold higher than the mutation rate in resting cells. Furthermore, DNA repair mechanisms, by which a cell maintains the integrity of its genetic code, are less reliable for the mtDNA than for the nuclear DNA, and the mutation rate for mitochondrial DNA is ten- to twenty-fold higher than that of nuclear DNA. The lack of immune surveillance during in vitro cell manipulations and expansion is believed to let such antigens develop and allows for their unchecked amplification.

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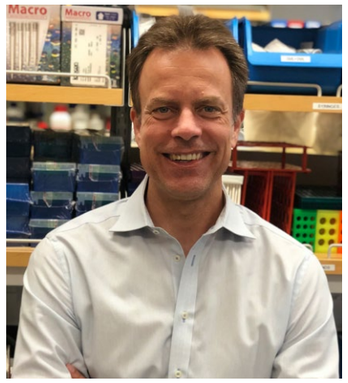
Stem cells hold great potential for treating a broad range of disease.

The mtDNA encodes only 13 protein subunits, but while a cell contains only two copies of its nuclear genome, it contains numerous mitochondria that each contain dozens of copies of their mitochondrial genome, which means that a cell can contain thousands of copies of its mitochondrial genome. Due to this high number of copies, mtDNA mutations may generate a high number of mutant proteins. Once an increasing prevalence of neoantigen burden crosses an activation threshold of the immune system, a cytotoxic response is triggered and derivatives of autologous iPSCs get



Dr Schrepfer and Dr Deuse's work is of critical importance in the area of stem cell transplantation as it provides a strategy to overcome cell rejection.

The team generated truly hypoimmune stem cells that evade immune rejection.

rejected. Dr Schrepfer and Dr Deuse's experiments in both mice and humans showed that the mutation of a single DNA base (a phenomenon called single nucleotide polymorphism, or SNP) is sufficient for the mutant protein to act as an antigen able to induce an immune response. Thorough quality control and routine screening for neoantigens during the manufacturing of autologous cell products is thus highly recommended. Reliable in vitro screening protocols have been described in Dr Schrepfer's and Dr Deuse's articles.

HYPO-IMMUNOGENIC iPSCS

Finally, Dr Schrepfer and Dr Deuse established a novel approach to overcome immune rejection by generating a new generation of immune-evasive iPSCs, named "hypoimmune iPSCs". Such iPSCs do not induce any cellular immune response because they are barely visible to the immune system. If such hypoimmune iPSCs could be generated, they could serve as a source for universally compatible cell and tissue grafts.

Their first strategy consisted of knocking down the expression of class I MHC in ESCs[7]. This knockdown resulted in less aggressive responses by the recipient immune system, and delayed the rejection of transplanted ESCs in mice. Although the work was performed before sophisticated gene editing was developed, it made the stem cell community start thinking about immune editing strategies. Those strategies include the modulation of activating and inhibitory surface molecules and release of tolerogenic factors. Several groups experimented with different immune editing approaches and confirmed the validity of this principle.

Dr Schrepfer and Dr Deuse examined syncytiotrophoblasts, cells from the placenta which form the interface between the maternal blood system and the foetus. The rationale behind this was that during pregnancy, the maternal body tolerates and even protects the developing foetus even though half of the antigens of the foetus originate from the father and are MHC mismatched. Pregnancy is a natural immune tolerance phenomenon that was used as a blueprint for a successful immune editing strategy.

The team observed that cells from the placenta expressed low levels of MHC antigens and high levels of CD47, a protein that inhibits a process named phagocytosis by which phagocytes (a type of immune cell) ingest target cells or particles. They used this knowledge to design hypoimmune iPSCs, which don't express MHC and have high expression levels of CD47. They employed gene editing to inactivate two genes crucial for class I (*B2M*) and class II (*CIITA*) MHC expression and used a lentivirus to add copies of the CD47 gene[7]. Both mouse and human hypoimmune iPSCs were generated and differentiated into various different cell types. Rigorous in vitro testing and additional in vivo cell transplants in mice confirmed that this immune editing strategy successfully silenced all types of lymphoid and myeloid immune cells and hypoimmune derivatives achieved long-term survival in fully MHC-mismatched recipients. Hypoimmune stem cell derivatives were thus moved into a development phase looking towards potential clinical applications.

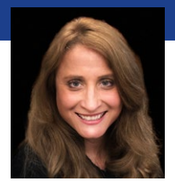
A NEW NK CELL IMMUNE CHECKPOINT IS DISCOVERED

While immune-edited cells from other groups that also included the elimination of class I MHC remained susceptible to natural killer (NK) cell killing, the immune editing strategy by Dr Schrepfer and Dr Deuse resulted in NK cell silencing and closed this gap for immune evasion. NK cells will inherently kill MHC-deficient target cells in the presence of inflammatory cytokines. Such cytokines can be released by other immune cells in response to the transplant procedure or the graft cell. In-depth analysis of NK cell interactions with immune-edited cells revealed a new immune checkpoint[8]. NK cells were shown to be able to upregulate the immune checkpoint receptor signal-regulatory protein-alpha, which delivers a strong inhibitory signal when engaging with its ligand CD47. An immune editing strategy including CD47 thus provides protection not only against phagocytes but also NK cells.

AN IMPACTFUL WORK

Dr Schrepfer and Dr Deuse's findings have been highlighted in leading journals such as *Nature* and *Science*[9-13]. Indeed, their work is of critical importance in the area of stem cell transplantation as it provides a strategy to overcome cell rejection. The generation of hypoimmune iPSCs can pave the way toward the manufacturing of universally compatible cell products for regenerative medicine, a goal commonly referred to as the "holy grail" of stem cell immunobiology. This platform technology was exclusively licensed to Sana Biotechnology Inc, of which Dr Schrepfer is scientific founder and Head of the Hypoimmune Platform to make stem cell-derived therapies available to anyone, anywhere, at any time.

Behind the Research



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W: https://adultctsurgery.ucsf.edu/conditions--procedures/minimally-invasive-mitral-valve-surgery.aspx

References

1. Swijnenburg R. J., Schrepfer S., Cao F., Pearl J. I., Xie X., Connolly A. J., Robbins R. C., & Wu J. C. (2008). In vivo imaging of embryonic stem cells reveals patterns of survival and immune rejection following transplantation. *Stem cells and development*, 17(6), 1023–1029. https://doi.org/10.1089/scd.2008.0091
2. Swijnenburg R. J., Schrepfer S., Govaert J. A., Cao F., Ransohoff K., Sheikh A. Y., Haddad M., Connolly A. J., Davis M. M., Robbins R. C., & Wu J. C. (2008). Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proceedings of the National Academy of Sciences of the United States of America*, 105(35), 12991–12996. https://doi.org/10.1073/pnas.0805802105
3. Hu X., Kueppers S., Kooreman N. G., Gravina A., Wang D., Tediashvili G., Marcus S., Fuchs S., Velden J., Reichenspurner H., Volk H. D., Deuse T. and Schrepfer S. (2020). H-Y- Incompatible Embryonic Stem Cell Transplantation causes rejection of nucleus matched cells. *Stem cells and development*. 29(18), 1179-1189. https://doi.org/10.1089/scd.2019.0299
4. Deuse T., Wang D., Stubbendorff M., Itagaki R., Grabosch A., Greaves L. C., Alawi M., Grünewald A., Hu X., Hua X., Velden J., Reichenspurner H., Robbins R. C., Jaenisch R., Weissman I. L., & Schrepfer S. (2015). SCNT-derived ESCs with mismatched mitochondria trigger an immune response in allogeneic hosts. *Cell stem cell*, 16(1), 33–38. https://doi.org/10.1016/j.stem.2014.11.003
5. Deuse T., Hu X., Agbor-Enoh S., Koch M., Spitzer M. H., Gravina A., Alawi M., Marishta A., Peters B., Kosaloglu-Yalcin Z., Yang Y., Rajalingam R., Wang D., Nashan B., Kiefmann R., Reichenspurner H., Valantine H., Weissman I. L., & Schrepfer S. (2019). De novo mutations in mitochondrial DNA of iPSCs produce immunogenic neoepitopes in mice and humans. *Nature biotechnology*, 37(10), 1137–1144. https://doi.org/10.1038/s41587-019-0227-7
6. Deuse T., Seifert M., Phillips N., Fire A., Tyan D., Kay M., Tsao P. S., Hua X., Velden J., Eiermann T., Volk H. D., Reichenspurner H., Robbins R. C. and Schrepfer S. (2011). Human Leukocyte Antigen I Knockdown Human Embryonic Stem Cells Induce Host Ignorance and Achieve Prolonged Xenogeneic Survival. *Circulation*. 124:S3-S9. https://doi.org/10.1161/CIRCULATIONAHA.111.020727
7. Deuse T., Hu X., Gravina A., Wang D., Tediashvili G., De C., WO. T, Wahl A., Garcia J., Davis M. M., Lanier L. L. and Schrepfer S. (2019). Hypo-immunogenic iPSC-derivatives show long-term survival in fully immunocompetent allogeneic recipients. *Nature Biotechnology*, 37(3):252-258. https://doi.org/10.1038/s41587-019-0016-3
8. Deuse T., Hu X., Agbor-Enoh S., Jang M.K., Alawi M., Saygi C., Gravina A., Tediashvili G., Nguyen V.Q., Liu Y., Valantine H., Lanier L.L., Schrepfer S. The SIRPα-CD47 immune checkpoint in NK cells. (2021). *J Exp Med.* ;218(3):e20200839. doi: 10.1084/jem.20200839.
9. Matched stem cells still rejected. (2014). *Nature*, 516:11.
10. Dunham-Snary K. J. and Ballinger S. W. (2015). Mitochondrial-nuclear DNA mismatch matters. *Science*, 349:1449-50.
11. European Perspectives. 2012. *Circulation*, 125:f37-f47.
12. European Perspectives Highlights 2012. *Circulation*, 2012:145-150.
13. European Perspectives in Cardiology. 2012. *Circulation*, 2012:37-47.

Research Objectives

Dr Schrepfer and Dr Deuse demonstrated that allogeneic and autologous cell grafts can induce an immune response and developed a new generation of cells to overcome immune rejection.

Detail

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Bio

Sonja Schrepfer, M.D., Ph.D. is Professor of Surgery at UCSF and founder of the Transplant and Stem Cell Immunobiology (TSI) Lab.

Tobias Deuse, M.D. is Professor of the Division of Adult Cardiothoracic Surgery at UCSF. He holds the Julien I.E. Hoffman, M.D. Endowed Chair in Cardiac Surgery. He is the director of the Minimally invasive Cardiac Surgery Program and the surgical director of the Transcatheter Valve Program.

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Disclosure:

S.S. is scientific founder and Senior Vice President of Sana Biotechnology Inc. and T.D. is scientific co-founder and advisor. Neither a reagent nor any funding from Sana Biotechnology Inc. was used in the described studies. UCSF has filed patent applications that cover these inventions.

Personal Response

What are the next steps for your line of hypoimmunogenic iPSCs to be used for therapeutic transplantation?

"Since the development of this technology, it has undergone thorough testing in several small and large animal models as well as human *in vitro* immune assays. The platform has been shown to very reliably generate immune evasive stem cells as well as differentiated tissue cells. Now, the technology is being translated from research grade cells to clinical grade cell products to address requirements for use in human clinical trials. We expect the first human studies to start enrolling following the submission of an IND, which submission is expected as early as 2022."