

**The immunogenicity of stem cells – a barrier to overcome
or
The immunogenicity of stem cells – a hurdle to jump over**

Kathryn J Wood¹, Fadi Issa² and Joanna Hester³

Transplantation Research Immunology Group
Nuffield Department of Surgical Sciences,
University of Oxford,
John Radcliffe Hospital,
Oxford OX3 9DU
UK

¹ Corresponding author – kathryn.wood@nds.ox.ac.uk

² FI is an Academic Clinical Lecture in Plastic Surgery

³ JH is a Kidney Research UK Senior Research Fellow

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Abstract

Stem cells and their differentiated progeny offer great hope for treating disease by providing an unlimited source of cells for repairing or replacing damaged tissue. Initial studies suggested that, unlike 'normal' transplants, specific characteristics of stem cells enabled them to avoid immune attack. However, recent findings have revealed that the immunogenicity of stem cells may have been underestimated. Here we review the current understanding of the mechanisms of immune recognition that are associated with stem cell immunogenicity. We discuss the relevance of reprogramming and differentiation strategies used to generate cells or tissue from stem cells for implantation in eliciting an immune response, examine the effectiveness of current strategies for minimizing immune attack in light of experience in the transplantation field, and outline important challenges moving forward.

Stem cell immunogenicity

Stem cell therapies offer enormous promise for treating disease but their potential immunogenicity is a critical issue that needs to be considered and a question that continues to challenge the field. Transplantation of any type of cell brings with it the possibility of triggering an immune response. Only if the cell implanted is absolutely identical to the recipient in every respect, including epigenetically, will it evade immune recognition.

The source of a stem cell as well as the evolution of technologies for both producing and differentiating them into the required tissue for therapy (Box 1) have each brought claims and counter claims regarding their immunogenicity or lack thereof. Understanding the scale and scope of the immunogenic challenge posed by different sources of stem cells and their differentiated progeny as well as the mechanisms used by the immune system to mount an attack will be essential for the effective and safe clinical translation of these novel therapies. Recent reports that pluripotent stem cells generated by somatic nuclear transfer (SCNT) can trigger an immune response [1] and that the immune response to tissues differentiated from the same induced pluripotent stem cell (iPSC) can vary depending on the type of cell produced [2] have rekindled the debate. Here we review recent advances on the mechanisms by which the immune system can recognise and respond to stem cells generated via distinct approaches, but with the common goal of therapy. We examine the impact of how stem cells are differentiated and transplanted on immunogenicity. Within this context, we discuss whether strategies that have been developed to prevent transplant rejection can be applied towards improving the clinical translation of stem cell therapies, and outline important considerations and as yet, unanswered questions in this regard.

Just how different from the host tissue are stem cells and their differentiated progeny?

ESCs derived from an individual unrelated to the patient requiring treatment clearly have the potential to be immunogenic as they will be allogeneic. ESCs and their derivatives can express variable, albeit low levels of major histocompatibility complex (MHC) antigens [3-5]. While it has been argued that this low-level expression of MHC class I (MHC-I) molecules may avoid T cell recognition, it is nevertheless a potential threat to the survival of the stem cells in vivo as instead they could be recognised and susceptible to killing by NK cells. Indeed, in vitro studies show that undifferentiated ESCs are targeted for lysis by NK cell [6] and that the absence of NK cells in vivo in SCID^{beige} mice allowed tumours, so-called teratomas, derived from mouse ESCs to grow much faster than in NK sufficient SCID mice [7]. MHC expression can be increased during differentiation of ESCs and in the presence of cytokines, such as interferon γ released during an immune response [8, 9]. While this may protect them from NK cell lysis, as was demonstrated for ESC-derived vascular progenitor cells [10], it will make them visible to T cells via essentially the same mechanisms that make allogeneic cell or tissue transplants susceptible to rejection (Figure 2; Box 2) [11], with so-called indirect presentation most likely playing a relatively more important role due to the low number of antigen presenting cells present.

Strategies to develop banks of ESCs may allow for matching of MHC/HLA molecules to overcome this potential source of immunogenicity (see below), but genetic differences in the expression of minor histocompatibility (miH) antigens between the donor and recipient are still likely. Clinical experience in bone marrow and haematopoietic stem cell (HSC) transplantation demonstrates that these miH mismatches are capable of triggering an immune response [12]. The acute rejection of mouse ESCs differentiated into embryoid bodies transplanted between MHC matched inbred mice that differed in expression of miH antigens demonstrates that this might also be the case for stem cells therapies [13]. Deuse and colleagues [1] generated NT-ESCs using enucleated oocytes from B6D2F1 (C57BL/6 \times DBA/2; H2^{bxd}) mice and nuclei from fibroblasts isolated from BALB/c (H2^d) mice.

The NT-ESCs therefore contained mitochondria of the oocyte donor expressing C57BL/6 (H2^b) miH antigens that were sufficient to initiate T helper cell activation as measured by IFN γ production in an ELISPOT assay and to trigger a Th1 response leading in vivo. Interestingly, the mice that made stronger Th1 responses either rejected the transplanted NT-ESCs or slowed their rate of growth. Purification of the mitochondria confirmed that they were the source of the immunogenicity and demonstrated that they alone stimulated the same immune response as the intact NT-ESCs. The authors hypothesise that indirect presentation of the mitochondrial miH antigens following antigen processing and conventional MHC presentation stimulated the Th1 response observed (Fig 2). These mouse studies highlight the potential immunogenicity of miH antigens that could be mismatched between the stem cell donor and patient.. Although no studies investigating this issue have been reported using human stem cells as yet, it is worth noting that the heterogeneity in human mitochondria is higher than in inbred mice [14] suggesting that this will require investigation. However, whether this is a barrier to therapy or a hurdle to be overcome will depend on the context and risk vs perceived benefit. For example, this strategy for generating stem cells would offer an advantage for the treatment of patients with inherited or acquired mitochondrial DNA diseases [15] and immunogenicity could be overcome by the use of immunosuppression or immune modulation (discussed below).

Stem cells generated from autologous somatic cells, ie the patient's own cells, by nuclear reprogramming (iPSCs; Box 1) clearly avoid the issues of genetic histo-incompatibility highlighted above and therefore should lack immunogenicity. However, it came to light that this may not always be the case when a study in mice using a teratoma model showed that undifferentiated iPSCs could trigger an immune response through residual expression of embryonic antigens due to incomplete reprogramming or ectopic expression through reprogramming and/or in vitro culture [16, 17]. These findings were controversial stimulating debate [18] and importantly, more experiments. Other groups investigating the immunogenicity of undifferentiated syngeneic mouse iPSCs in vivo did not find them to be immunogenic [19] and after differentiation the immunogenicity of iPSCs has been reported to decrease the closer the surface antigen expression profile becomes to that of the parent somatic cells [20]. Moreover, in vivo in mice the immunogenicity of iPSCs was found to be negligible following differentiation into tissues such as skin, bone marrow, hepatocytes or neuronal cells [21, 22]. Data from a large animal model where autologous iPSCs derived differentiated cells have been demonstrated to be functional [23] and to survive in vivo in non-human primates support these findings. For example in a model of Parkinson's disease autologous iPSC derived dopamine neurons implanted into the brain were found to survive and function for up to 2 years without immunosuppression [24], while in another study they were reported to trigger only a minimal immune response compared to allogeneic cells [25]. These findings are very encouraging, but also highlight the complexity of the issue regarding the immunogenicity of stem cells and raise many additional questions. For example, are the differentiation protocols developed to date sufficiently robust to ensure complete differentiation of all the cells in the culture and therefore the lack of potential immunogenicity? If not, is it possible to purify the differentiated cells away from any unwanted cells such as undifferentiated iPSCs or other types of cells arising in the cultures? For example as has been shown possible for iPSC derived dopaminergic neurons using cell sorting [26].

In addition to the potential for residual expression of embryonic antigens, reprogramming using either viral or non-viral plasmid based approaches has been reported to alter expression of a multitude of other genes. The culture conditions required to produce iPSCs and the length of time the cells remain in culture during differentiation could result in epigenetic changes and expression of new molecules with the capacity to elicit an immune response. Residual gene expression of the donor cell type used to generate human iPSCs has been found to contribute to the differences among hiPSCs to add to the incompleteness in reprogramming [27]. Awe and colleagues used

correlative gene expression analysis of the two putative mouse “immunogenicity” genes, zymogen granule protein 16 (*Zg16*) that can play a role in protein trafficking in the cell and HORMA domain-containing protein 1 (*Hormad1*) that plays a role in cell division [16], to assay the expression levels of the human homologous in human ESCs and iPSCs [28]. *ZG16* expression was found to be heterogeneous amongst both stem cell types, but when it was expressed ectopically in human APCs it was insufficient to trigger a detectable response in a PBMC coculture assay. At face value these data suggest that this molecule may not be immunogenic in humans, however, it is important to remember the context in which a potential antigen is recognised by the immune system will dictate how the response evolves, and in vitro assays may fail to reflect accurately the inflammation induced at the site of implantation. Culture conditions were found to impose distinctive transcriptional and epigenomic properties on mouse ESCs [29]. Large scale analyses of a panel of human iPSCs clones maintained and differentiated into neuronal cells under the same conditions, demonstrated that some clones can retain a significant number of undifferentiated cells after differentiation [30]. Moreover, the ‘differentiation defective’ clones formed teratomas when transplanted into mouse brains. Analysis of the molecular signatures of the differentiation defective clones revealed that they some exhibited an aberrant epigenetic status and with high expression levels of a number of genes including those expressed from the long terminal repeats (LTRs) of specific human endogenous retroviruses. In fully differentiated clones, the LTRs were silenced by a variety of epigenetic modifications including DNA methylation whereas the differentiation defective exhibited lower levels of methylation [30]. Changes in epigenetic profiles associated with hypomethylation have also been reported for other types of stem cell. For example, culture of human ESCs was also found to promote the epigenetic expression of CD30, a biomarker for malignant cells in Hodgkin's disease and embryonal carcinoma cells [31]. CD30 expression in this study was traced back to the levels of ascorbate in the media used to culture the human ESCs, and occurred as a result of a dramatic loss of DNA methylation of a CpG island in the CD30 promoter.

The probability that all iPSC clones may not behave in an identical fashion during differentiation suggests that it will be important to determine if undifferentiated iPSCs are still present. The use of high resolution genome wide sequencing and global transcriptome analysis to profile to compare different cells produced from the same or different stem cell sources under the same or different conditions is likely to be an important tool. Further work is clearly required to determine why these epigenetic abnormalities can occur, how they relate to the defective differentiation observed and whether they have functional consequences to ensure that they can be eliminated before clinical translation.

The type of cell differentiated from autologous iPSC may also affect its potential immunogenicity in vivo. Humanised mouse models can be used to investigate the immunogenicity of different types of undifferentiated as well as differentiated human stem cells (eg [32]). Comparing the immune response to smooth muscle (SM) and retinal pigment epithelial (RPE) cells differentiated from the same human iPSC highlighted that while some cell types lack immunogenicity others may trigger rejection [2]. In this study autologous, fibroblasts were used to generate integration-free human iPSCs while the foetal liver from the same donor was used to reconstitute a human immune system in NOD/SCID/g null (NSG) mice. RPE cells differentiated from the iPSCs did not trigger an immune response when transplanted into these humanised mice, but the SM cells were immunogenic. Interestingly, the lack of immunogenicity of autologous non-human primate iPSC derived RPEs and human iPSC derived RPEs has also been reported in other studies [33, 34]. These data support the hypothesis that each cell type differentiated from stem cells could have a different level of immunogenicity and this will depend on many factors including epigenetic abnormalities as discussed above, the intrinsic immune characteristics of different cell types and the site of implantation (see below) amongst others.

A further potential source of stem cell immunogenicity that needs to be taken into account is the blood group antigens. Human ESCs both before and after differentiation into hepatocytes or cardiomyocytes have been demonstrated to express ABO antigens [35]. Clearly, it will be important to take into account blood group compatibility in clinical studies of stem cell therapy as implantation of ABO incompatible stem cells could trigger an immune response.

The absence of self tolerance is already known to be a barrier in other types of replacement therapy used to correct a gene defect. In this situation as neither the wild type gene nor its product were obviously present at the critical point during selection of the T and B cell repertoires tolerance was not induced. For example, conventional enzyme replacement therapy triggers the production of neutralizing antibodies that can significantly compromise the bioactivity of therapeutic enzymes [36]. For stem cell therapies, the absence of self tolerance to the wild type functional gene introduced into an autologous iPSC to correct a defect may also be immunogenic triggering an immune response that could compromise the survival of the implanted cells.

Mechanisms of immune attack

Does simply implanting stem cells in the patient contribute?

Any source of tissue damage, including the trauma/inflammation associated with the implantation of stem cells in vivo will contribute to the activation of an immune response. ESC-derived progenitors and iPSCs may express pattern recognition receptors such as the toll like receptors (TLRs) or be exposed inherent danger signals associated with tissue damage/injury (DAMPs) or to pathogen associated molecular patterns (PAMPS) at some stage in either their manipulation or implantation. While this early inflammatory response may resolve relatively quickly in the absence of other triggers its significance to the overall character of the immune response that is triggered will depend on a number of factors including the site of implantation, the amount of tissue disruption created by the procedure and critically whether antigens that can be recognised by the immune system are present as discussed above. As yet, few studies have examined the contribution these early innate responses following stem cell implantation in detail.

Differences in TLR expression and TLR dependent responses have been characterised between ESCs, ESC differentiated into cardiomyocytes or endothelial cells (ECs) and human aortic ECs themselves [37]. In general, TLR expression levels were lower on ESCs than human aortic ECs, whereas differentiated ESC-derived cardiomyocytes expressed higher levels which increased over time in culture. While human aortic ECs displayed functional TLR signalling, ESCs or differentiated ESCs did not respond to a range of known pathogen associated molecular patterns, with the exception of TLR5, suggesting that they were largely non-functional [37]. Interestingly, there is evidence that TLR4 expression on ESCs and their differentiated derivatives is controlled epigenetically through methylation of the TLR4 gene promoter [38], thus the hypomethylation states observed in some differentiated iPSCs as noted above [30] may be the explanation. However, there are conflicting reports in the literature, for example Lee *et al* demonstrated a capacity for ESCs and ESC derivatives to sense and respond to microbial stimulation [39] through both TLR3 and TLR4. The divergence in these findings may be accounted for by the different culture and experimental conditions used. As mentioned above in relation to CD30 expression, the culture conditions themselves can contribute to epigenetic changes in stem cells and their differentiated progeny.

In our laboratory, we compared immune response mounted against insulin producing cell clusters (IPCCs) derived from mouse ESCs versus allogeneic mouse islet grafts in the first few days after transplantation. Based on analysis of the infiltration of innate immune cells, such as macrophages and neutrophils, and inflammatory chemokine expression, IPCCs were found to be less susceptible

than allogeneic islet grafts [40]. We did not compare DAMP expression in the 2 sources of cells, but we would hypothesise that the allogeneic islet grafts produced through a collagenase digestion procedure expressed higher levels. In addition, islet allografts in contrast to IPCCs differentiated *in vitro* contain passenger leukocytes that have inherent antigen presenting cell capacity thus allowing, after innate immune activation, presentation of the alloantigens to the host immune system via the direct pathway of allorecognition (Fig 2; Box 2) leading to an accelerated immune response, including T cell infiltration into islets grafts. Thus although the IPCCs triggered innate immunity after implantation, they were compromised in their ability to activate the adaptive immune system.

Another potential hurdle is the successful integration of the differentiated stem cells into the damaged target tissue where they are to function following implantation. In some cases, the underlying disease requiring treatment may have resulted in changes that could affect the way in which the introduced cells localise and function as well as how inflammatory mediators are produced when the tissue is disrupted during stem cell implantation. For example, in the brain, the disruption of the blood brain barrier that is necessary for the introduction of stem cells to the site of damage, would also allow the immune system unrestrained access to the site until the barrier reformed. In the non-human primate studies mentioned above, although sufficient functional activity of the autologous iPSC-derived dopamine neurones was detected 2 years after implantation [24], it is not possible to determine if the same number cells injected was still present. It is possible that there was an early inflammatory response in the brain [25] that targeted some of the injected cells but that this was self-limiting leaving sufficient functional cells to control disease in the longer-term. Injecting a higher number of cells and/or short-term control of the inflammatory response may be beneficial.

The orientation and the ability of the differentiated stem cells to produce a 3-dimensional, multi-cell structure may also be important. To address this latter issue biodegradable scaffolds have been developed to enhance integration, support natural repair mechanisms and/or produce bioactive molecules are being investigated in stem cell therapy applications [41]. The types of scaffolds being investigated in the context of stem cell therapies include polymer scaffolds that use materials with controlled degradation over the course of months and extracellular matrix derived scaffolds that are already used in the clinic for wound repair. Decellularised organ scaffolds that can be seeded with stem cells or autologous differentiated cells are an alternative approach under investigation. To date, cells combined with either a chemical scaffold or seeded into a decellularised organ have been used to repair tissues that have a structural purpose such as the ureter or trachea respectively. For example, a decellularized human trachea from a deceased organ donor seeded with autologous epithelial cells and human mesenchymal stromal cells (MSCs) has been used successfully to replace the trachea in a child with a congenital abnormality of the trachea [42]. These approaches may prove to be essential for the success of the stem cell therapy in some disease settings, but they may also present challenges in terms of contributing to the inflammatory environment at the site of implantation and to immune recognition as each modification of the stem cell and its environment could create new targets for the immune response. At present it is not known how, or indeed if, the interactions with the immune system will have an impact on clinical success.

Are T cells involved?

T cell responses have consistently been reported following implantation of ESCs, iPSCs and NT-ESTs due to changes in the expression of antigens that can be recognised by the immune system as summarised above. Th1 responses have been documented most frequently, but there is evidence that all elements of a T cell response can be involved, including CD8⁺ cytokine producing

and cytotoxic cells [1, 4, 8]. The pathway of stimulation has not been characterised in most cases, but as most patients requiring stem cell therapy will have a full complement of APCs, the indirect route of antigen presentation is likely to be the dominant pathway for stimulating a naïve T cell response to any miH antigens or abnormally expressed molecules in vivo (Fig 2; Box2). Stem cells that express mismatched MHC molecules could present the mismatched MHC molecules directly to the patient's T cells if they also expressed costimulatory molecules. While there is evidence that some stem cells can express molecules associated with APC function, such as CD86, CD80 and CD11c, expression levels are in general reported to be low on both mouse and human ESC and iPSC lines and do not appear to increase markedly in expression during differentiation and culture [5]. Thus direct allostimulation may be of only limited significance in the context of stem cell therapy. In contrast, for stimulation of memory T cells much lower levels of costimulation are required, therefore if memory T cells reactive to any of the molecules expressed by a stem cell are present in the patient, these could potentially be activated by the stem cell therapy and contribute to the immune response.

Strategies to overcome stem cell immunogenicity and minimise the impact of immune recognition and attack

HLA matching and manipulation of MHC expression

Creation of a human ESC bank of sufficient diversity with respect to HLA antigens that it would be possible to select a source of cells for therapy with a 'reasonable' HLA match to the patient requiring treatment has been suggested as a way of reducing the potential for the immune system to respond to ESCs [43]. This approach has proved very successful for bone marrow and haematopoietic stem cell (HSC) transplantation where international registries of HLA typed individuals who are willing to act as cell donors (www.worldmarrow.org) or HLA typed cord blood samples (www.bmdw.org) enable an HLA matched donor to be found for patients requiring a transplant. To predict how large an ESC bank would need to be, Taylor and colleagues used the UK population as a model and estimated that a bank of approximately 150 cell lines would provide an HLA match at 4 loci, HLA-A,B,C and DR, for approximately 20% of patients. Obviously, this sort of model needs to be interpreted in the light of the information now available regarding the extent of the immune response against stem cells from an autologous source as discussed above. Using stem cell banks or generating iPSCs from an HLA matched unaffected individual who have volunteered to become a member of a global network of iPSC haplobanks [44] would also overcome the inherent genetic defects that would be carried over by autologous cells in some disease settings.

Alternative strategies proposed for manipulating MHC expression to decrease the magnitude of an immune attack include knocking down or reducing MHC expression in stem cells for therapy may also decrease the magnitude of an immune attack [45]. However, as mentioned above, this may leave the cells open to killing by NK cells and indirectly could introduce other variations that themselves could trigger an immune response.

Controlling the unwanted immune responses

Some stem cells may express or produce immunomodulatory molecules providing themselves with some protection from immune attack by inhibiting the initiation or evolution of a T cell response such as is the case for either tissue derived or iPSC differentiated mesoangioblasts [46, 47].

Combinations of immunosuppressive drugs, both small molecules and biological agents, are used very successfully to prevent the acute rejection of cell and organ transplants (eg. [48]). Immunosuppressive drugs have been shown to prolong survival of mouse ESCs in the short term (eg. [49]) and should be considered as a strategy for controlling an unwanted immune response in

patients treated with stem cell therapy. However, the administration of immunosuppressive drugs over long periods of time brings with it significant risks for the well-being of the patient, including an increased susceptibility to viral infections and risk of cancer together with the inherent toxicity of the drugs themselves that can result in the development of diabetes and nephrotoxicity as is well documented in organ transplant recipients treated with the calcineurin inhibitors tacrolimus and cyclosporin. Unlike in organ transplantation where the risk-benefit analysis is clearly in favour of using life-long immunosuppressive drugs to control rejection [50], for stem cell therapy, particularly if the disease process to be treated is not life threatening, the risk-benefit profile may depend on the duration of immunosuppression required. A careful analysis would need to be undertaken on a case by case basis.

Costimulation and accessory molecule blockade

Another possible option for controlling the immune response to differentiated stem cells is block the interaction between accessory or costimulation molecules and their ligands [51]. Non-depleting monoclonal antibodies specific for the T cell coreceptors, CD4 and CD8 were demonstrated to prevent rejection and lead to tolerance to mouse ESCs differentiated into embryoid bodies [13]. Short term costimulation blockade using a combination of anti-CD40L, anti-LFA-1, and CTLA4-Ig also resulted in the survival of mouse and human iPSCs [49, 52, 53]. An alternative approach to blocking costimulatory molecules by administration of biological agents is for the stem cells to express or produce the immunomodulatory molecules themselves either naturally or through molecular engineering. Some stem cells have been reported to do this naturally, as is the case for tissue derived or iPSC differentiated mesoangioblasts [46, 47]. Rong and colleagues established knockin human ESCs that constitutively expressed CTLA4-Ig and PD-L1 before and after differentiation and demonstrated that this protected allogeneic hESC-derived teratomas, fibroblasts, and cardiomyocytes from rejection [32]. Expression of both CTLA4-Ig and PD-L1 was required to confer immune protection, as neither was sufficient on their own.

Regulatory T cells

Regulatory T (Treg) cells can be utilised to control unwanted immune responses either through the recruitment or expansion of thymus derived Treg or through generation of induced Treg following exposure to antigen [54]. Treg cells have been shown capable of controlling CD8⁺ T cell and macrophage responses mounted against an ESC-derived graft and data from our group have demonstrated the capacity for autologous *ex vivo* expanded Treg to prevent the rejection of human tissue in humanised mouse models including of human mesoangioblasts ([55-57]; Li and Wood personal communication). These findings suggest that it may be feasible to generate Treg in situ in the patient through treatments such as costimulation blockade [58] as mentioned above or from the stem cell donor to use as an adjunctive cellular therapy. Indeed functional Tregs with the expected phenotype have been differentiated from iPSCs by introducing the FoxP3 gene and ligating Notch ligand [59], demonstrated to secrete both TGF- β and IL-10 in vivo and in vitro and to suppress host immune responses including reducing arthritis development in mouse models.

Strategies to induce immunological unresponsiveness

The mechanism underpinning the induction and maintenance of both central and peripheral tolerance that have been utilised to develop strategies induce tolerance to donor alloantigens in transplant recipients [60] and can also be applied to stem cell therapy. Deuse and colleagues demonstrated that an iv infusion of mitomycin-treated mitochondrial antigen mismatched NT-ESC into new-born BLAB/c mice would induce tolerance to the maternal mitochondrial antigens such that the adult BALB/c mice no longer rejected the NT-ESC grafts [1]; a strategy based on the landmark studies of Billingham, Brent and Medawar. Lessons learnt from the development of tolerance strategies in adult organ transplant recipients may help guide the stem cell field. Such strategies could include the use of immune regulatory cells to control unwanted immune responses

[54]; the induction of either mixed chimerism, ie the co-existence of donor and recipient haematopoietic cells in HLA matched and haplo-identical kidney transplant recipients eg. [61-63] or full chimerism in HLA mismatched kidney transplant recipients [64]. In each case the recipients require only a limited period of non-specific immunosuppressive drug therapy to allow in the longer-term the survival and function of the kidney transplant in the absence of immunosuppressive drugs, but the conditioning required to enable stem cell engraftment is significant. Thus whether these strategies for the induction of acquired tolerance are applicable and would be safe and effective to use in the context of stem cell therapy remains to be fully investigated.

Using the stem cells themselves to differentiate a source of cells that would induce tolerance or regulate the immune responses to a subsequent stem cell therapy as in the case of stem cell derived regulatory T cells outlined above is interesting. Fairchild and colleagues have proposed that using immature dendritic cells (DCs) differentiated from the same source of stem cells as the tissue required for therapy and therefore expressing the antigens to which tolerance is required could offer a solution. Immature DCs express low levels of MHC and co-stimulatory molecules and their administration in vivo and in the absence of inflammation has been shown to invoke immune regulatory networks including the generation of Treg cells [65], as mentioned above. DCs have been differentiated from mouse [66] and human iPSCs [67] and in mice, indefinite survival of skin grafts across a miH barrier was achieved using immature donor iPSC-derived DCs [68]. Differentiation of other types of cells from stem cells, such as HSCs, may also provide an approach for inducing tolerance as demonstrated by the induction of tolerance to islet allografts in NOD mice using haematopoietic stem cells differentiated from MHC mismatched ESCs [69].

Concluding Remarks

Immunogenicity has emerged as a significant problem not only with allogeneic ESCs, but also potentially with autologous stem cell sources including iPSCs. Genome wide screening and transcriptome profiling will facilitate the identification of clonal differences enabling a systematic evaluation of the potential immunogenicity of the stem cells to be used for therapy in the context of the disease and the individual patient being treated. This together with improved in vivo models will allow a careful evaluation of potential immunogenicity. If stem cell therapies do trigger an immune response then there are many strategies available for controlling such unwanted responses that could compromise the efficacy of the treatment. While the risks associated with the long-term use of non-specific immunosuppressive drug therapy may outweigh the benefits of stem cell therapy in some disease settings, this will not be the case for all and the development of short term strategies to overcome the acute immune response to stem cells mismatched for only a limited number of miH antigens immediately after implantation may be sufficient to invoke natural immune regulatory mechanisms that will allow the cells to survive and function long-term.

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Box 1.

Sources of stem cells, their levels of potency and immunogenicity

- Embryonic stem cells (ESCs) are derived from the inner cell mass of a blastocyst. They are pluripotent possessing the ability to give rise to all of the cells of the embryo ie to all 3 germ layers.
ESCs are most likely from an unrelated donor and may express mismatched MHC and/or miH antigens that could trigger an immune response.
- Nuclear transfer derived embryonic stem cells (NT-ESC) are pluripotent stem cells generated by transplanting the nucleus of a somatic cell into an enucleated oocyte – referred to as somatic cell nuclear transfer (SCNT).
NT-ESCs contain allogeneic mitochondria that are immunogenic
- Induced pluripotent stem cells (iPSCs) are adult differentiated somatic cells that have been undergone nuclear reprogramming reverting them to a pluripotent state. They can be produced from the individual requiring therapy ie they are autologous. There is no age limit. An unlimited supply of cells can be produced.
Undifferentiated iPSCs may express molecules of embryonic origin associated with reprogramming that may trigger an immune response while fully differentiated cells demonstrate lower levels of immunogenicity.
- Haematopoietic stem cells (HSCs) are multipotent, found in the bone marrow and have the ability to differentiate into different cell types within the haematopoietic lineage.
HSCs differentiated from the stem cell donor could be used to induce tolerance

Figure 1. Sources of stem cells for therapy (adapted from [70])

Box 2

Pathways of immune recognition by T cells and their relevance to stem cell therapy

- **Direct pathway** – recognition of intact mismatched MHC molecules by host T cells usually triggered by donor derived antigen presenting cells that express costimulatory molecules.

As cells and tissues differentiated from stem cells are unlikely to have a significant APC content, this pathway is unlikely to play a major role in triggering an immune response to stem cell derived tissues in vivo.

- **Indirect pathway** – recognition of allopeptides derived from MHC or miH antigens including those induce by epigenetic changes by host APCs to T cells ie the physiological pathway of T cell recognition.

Likely to be the dominant pathway for triggering a T cell response to stem cell therapy.#

- **Semi-direct pathway** – the acquisition of MHC molecules through membrane exchange by host APC.

This pathway could play a role in triggering a response to stem cells but there is no evidence that this is the case as yet.

Figure 2. Pathways of alloantigen recognition