Immunological considerations for cell therapy using human embryonic stem cell derivatives

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Abstract

The isolation of human embryonic stem cells (hESCs) and their directed differentiation into a variety of tissue specific cell types offers a novel approach for production of cellular transplants. Still, successful translation of the therapeutic promise of hESCs to clinical practice depends heavily on purification of beneficial cell populations as well as on prevention of immune rejection. This review focuses on the immunological antigens that are expressed on differentiated progenies of hESCs and on the adaptive and innate immune processes that may target their implanted derivatives. The risk of immune rejection has driven the development of methods that may be used for preparation of patient-specific histocompatible pluripotent cell lines. It is hoped that pluripotent cell lines that will be generated following somatic cell nuclear transfer into oocytes or zygotes, oocyte parthenogenesis or introduction of reprogramming factors will be largely protected from immune rejection. These techniques are presented and the remaining immunological challenges for creating tolerated implants using these approaches are discussed.


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1. Introduction

Since the early days of modern medicine it has been known that transplantation of tissues from one individual to another results in immune rejection, except when performed between identical twins. Extensive research done in the past decades has uncovered that differences in protein sequences between individuals are the primary targets for the immune response against allogeneic (genetically non-identical) grafts. These molecules were subsequently defined as histocompatibility antigens as they determine whether implanted cells are accepted or rejected by the immune system. Today, the risk of organ rejection remains high despite efforts to match haplotypes of histocompatibility antigens as well as use of immunosuppressive therapies.

Human embryonic stem cells (hESCs) can be propagated in high numbers in vitro, and have the potential to differentiate into a myriad of cell types (Reubinoff et al., 2000; Thomson et al., 1998). The application of these cells for organ restoration is of particular importance due to limited availability of donated organs. However, it is unclear whether differentiated hESCs will be recognized by the immune system following implantation in humans. Therefore it is imperative to investigate the immune response against differentiated hESCs for successful clinical translation.

The purpose of this review is to describe the current knowledge of antigenicity and immunogenicity of hESCs and their derivatives. While antigenicity refers only to the capacity of cells to express histocompatibility antigens, immunogenicity is a broader term for the potential to elicit immune response through additional factors, such as co-stimulatory molecules. This review summarizes our current view of the histocompatibility antigens and immunomodulating molecules that are expressed on undifferentiated and differentiated hESCs (u/dhESCs). In addition, adaptive and innate immune processes that may develop against the cells following recognition of histocompatibility antigens will be discussed. As rejection processes could severely limit the use of hESC-derived transplants, multiple approaches are being developed to alleviate these risks. I will focus on techniques for derivation of isogenic (genetically identical) hESC lines and discuss the immunological benefits and remaining challenges for their potential application.

2. Expression of histocompatibility antigens on u/dhESCs

Histocompatibility antigens are classically divided into major histocompatibility complex (MHC) molecules, minor histocompatibility complex antigens (mHAg; Roopenian et al., 2002) and ABO blood group antigens (Watkins, 2001). The prominent mechanism of graft rejection is initiated by recognition of the graft’s MHC molecules by host T cells (Lechler et al., 2005). Under normal circumstances, MHC molecules present processed cellular and extra-cellular antigens to circulating T cells. Since T cells that recognize self-antigens are depleted in the thymus, circulating T cells respond only to foreign antigens bound by MHC molecules. This situation changes dramatically following transplantation of cells expressing allelic variants of MHC proteins to unmatched recipient. Due to similarity in structure, foreign MHC molecules may interact with the recipient’s T cells, evidently “mimicking” self-MHC molecules presenting foreign antigens (Suchin et al., 2001). As a result, “direct allorecognition” (see Figure 1A) between host T cells and transplanted cells leads to sensitization, proliferation and cytotoxic response against the graft (Morelli and Thomson, 2003). This response is initiated primarily by “passenger” dendritic cells (DCs) that migrate from transplanted organs to lymph nodes of the recipient where they allostimulate T cells. Importantly, the initiation, propagation and termination of direct alloresponse depend also on additional stimulatory and inhibitory signals provided by the transplanted cells as well as the site of transplantation and extent of graft vascularization (discussed below). Still, it is predominantly acute and unless treated with immunosuppressive drugs results in rapid graft failure.

Although immunosuppressive therapy slows immune rejection processes, a large proportion of tissue grafts succumb to chronic rejection that results in graft failure. Chronic immune responses are thought to result primarily from processing of donor antigens by the recipient’s or the donor’s professional antigen presenting cells (APCs), such as DCs and macrophages, and their presentation to T cells (Briscoe and Sayegh, 2002). This “indirect allorecognition” (see Figure 1B) pathway is largely analogous to physiologic antigen processing and is assumed to mediate graft rejection mainly by generation of alloantibodies and allospecific cytotoxic T cells (Bolton et al., 2008). As virtually all circulating antigens are ingested and processed by APCs, chronic immune processes could target numerous proteins that differ in structure between donor cells and the recipient. The majority of these processes are aimed at MHC proteins due to their extreme polymorphism, but any other polymorphic antigens, collectively known as mHAg, may be targeted.

To reduce the immune response against allografts, efforts are being made to match as many MHC alleles as possible prior to organ transplantation. However, it is particularly hard to match MHC haplotypes between genetically
Unrelated individuals since there are up to several hundreds alleles encoding for the three MHC class I (MHC-I) genes and four pairs of MHC class II (MHC-II) genes. Both MHC classes are known to affect transplantation outcomes, since MHC-I molecules are ubiquitously expressed and MHC-II expressing hematopoietic and DCs are present in most tissues.

To evaluate the antigenic properties of u/d hESCs, the cell-surface expression of both MHC classes was tested via fluorescence-activated cell sorter (FACS). It was found that undifferentiated cells express relatively low levels of MHC-I molecules (Draper et al., 2002; Drukker et al., 2002) and do not express MHC-II molecules (Drukker et al., 2002), similarly to the inner cell mass (ICM) of human blastocyst stage embryos from which ESC are derived (Jurisicova et al., 1996). When treated with interferon-γ (IFN-γ), a pro inflammatory cytokine, MHC-I levels were substantially upregulated while MHC-II molecules remained undetected. Aggregation induced differentiation using embryoid body (EB) formation caused approximately 10-fold increase in MHC-I expression on the cell surface, and application of IFNs induced approximately 100-fold increase. Furthermore, MHC-I expression levels reached somatic levels on
Figure 1. Proposed pathways of interaction between transplanted hESC derivatives and the immune system continued. (C). Natural killer (NK) cell activation is controlled by integration of signals from activatory and inhibitory receptors. Inhibitory NK cell receptors recognize self-MHC-I molecules that restrain their activation (left). When unimpeded by the inhibitory receptors, binding of NK cell activation receptors to their ligands on target cells results in NK cell stimulation (right). (D) As most differentiated hESCs do not express co-stimulatory molecules (right) they may be protected from direct allore cognition (but their antigens can presumably initiate chronic immune responses through the indirect pathway). When MHC-I expression is low and lyss ligands are absent, hESC derivatives may be protected from NK-cell response (left). Cardiomyocytes derived from hESCs could potentially be used for restoration of heart functions (center). TCR T cell receptor.

IFN-γ treated teratoma cells (Drukker et al., 2002). MHC-II molecules remained below detection level under these conditions, however, other differentiation protocols have reported expression of these molecules, especially during hematopoietic and DC differentiation (Senju et al., 2007; Slukvin et al., 2006; Zhan et al., 2004). These data suggest that differentiated hESCs would express high levels of MHC-I following transplantation, while only a small number of differentiation protocols could promote expression of MHC-II (see Table 1).

The status of mHAgs expression on u/dhESCs has not been directly examined to date, but their expression is expected. One group of mHAgs, which could be easily avoided by transplanting male hESC lines only to males, are derived from genes encoded by the Y chromosome, termed the HY antigens (Wang et al., 1995). It is also reasonable that matching ubiquitously expressed mHAgs such as, HA-3, -4, -6, -7 and -8 (Brickner et al., 2001; de Bueger et al., 1992) would improve survival of hESC-derived grafts and in cases where hESC-derived hematopoietic stem cells are transplanted, HA-1 and -2 should be matched as they are restricted to cells of this lineage (de Bueger et al., 1992). In addition, although the mitochondrial genome is relatively small, efforts should be made to match mitochondrial haplotypes that were shown to encode for mHAgs (discussed below). Finally, it is important to investigate whether hESC-derived embryonic antigens would constitute an additional class of mHAgs to which recipients are not tolerant, since they are not significantly expressed in adults. One group of histocompatibility antigens that was not yet examined in u/dhESCs is the ABO blood group antigens. Since matching can easily prevent immune response against ABO antigens, it will not be discussed here further.

3. Expression of T-cell regulating signals in u/dhESCs

Apart from MHC interaction, co-stimulatory molecules are vital signals for the initiation and propagation of T-cell mediated immune responses. The CD80 and CD86 (B7.1 and B7.2, respectively) ligands are of special importance
Table 1. Expression of histocompatibility antigens and immune regulatory molecules in hESCs and their differentiated derivatives

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Undifferentiated cells</th>
<th>Differentiated cells</th>
<th>Role in alloresponse</th>
</tr>
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<tbody>
<tr>
<td>MHC-I molecules</td>
<td>Low (Draper et al., 2002; Drukker et al., 2002)</td>
<td>High (Draper et al., 2002; Drukker et al., 2002)</td>
<td>Activates T cells, repress NK cells</td>
</tr>
<tr>
<td>MHC-II molecules</td>
<td>(Drukker et al., 2002)</td>
<td>Hematopoietic and DCs (Senju et al., 2007; Slukvin et al., 2006; Zhan et al., 2004)</td>
<td>Activates T cells</td>
</tr>
<tr>
<td>HLA-G</td>
<td>(Drukker et al., 2002)</td>
<td>Trophoblasts (Xu et al., 2002)</td>
<td>Represses NK cells</td>
</tr>
<tr>
<td>HLA-E (Drukker et al., 2002)</td>
<td>Low</td>
<td>High</td>
<td>Represses NK cells</td>
</tr>
<tr>
<td>NK-cell lysis ligands (Drukker et al., 2002)</td>
<td>-</td>
<td>Hematopoietic and DCs (Senju et al., 2007; Slukvin et al., 2006; Zhan et al., 2004)</td>
<td>Activates T cells</td>
</tr>
<tr>
<td>CD80 and CD86</td>
<td>(Drukker et al., 2006)</td>
<td>-</td>
<td>Activates T cells</td>
</tr>
<tr>
<td>Fas L (Drukker et al., 2006)</td>
<td>-</td>
<td>-</td>
<td>Represses leukocytes</td>
</tr>
</tbody>
</table>

as they are necessary for binding the activatory receptor CD28 on T cells (Lechler et al., 2005). These molecules are primarily expressed on activated APCs and in conjunction with indirect or direct alloantigen presentation they have a central role in the graft rejection response (see Figure 1A and B). Analysis of co-stimulatory molecules on u/dhESCs showed that undifferentiated cells do not express these molecules (Drukker et al., 2006), however, differentiated cells could express them following commitment to the hematopoietic lineage and DC differentiation (see Table 1; Senju et al., 2007; Slukvin et al., 2006; Zhan et al., 2004). Therefore, transplantation involving hESC-derived hematopoietic cells carries high risks for direct co-stimulation of host T-cells. The presence of endothelial cells derived from hESCs could further intensify this response since they could directly or indirectly co-stimulate T cells (Kreisel et al., 2002). Other molecules involved in co-stimulation of T cells include the adhesion molecules intercellular adhesion molecule (ICAM) -1 and -3 as well as the recently identified members of the B7 family (B7-H1, -H2 and -H3; Suh et al., 2003). However, at this point it is unknown whether differentiated hESCs express them.

Contrary to stimulatory molecules, several ligands have inhibitory effect on T cells. One of the best-characterized interactions is the Fas ligand (FasL) which is recognized by lymphocytes expressing the Fas receptor (death receptor; see Figure 1A). Activation of Fas induces apoptosis in lymphocytes and is known to have numerous roles in the termination of T cell responses and restriction of immune responses in immune-privileged sites of the body, such as the cornea and testis (Green and Ferguson, 2001). Although it was shown that expression of FasL on rat embryonic stem-like cells correlates with protection from allograft rejection (Fandrich et al., 2002), expression of this molecule on hESCs was not detected (see Table 1; Drukker et al., 2006). It was also shown that mouse ESCs express functional Serpin-6 (Abdullah et al., 2007), an inhibitor of cytotoxic T lymphocytes; however, at this point it is unknown whether hESCs express it too. There are several claims that u/dhESCs may possess immune-privileged properties (Li et al., 2004), but the molecular pathways that are involved were not identified. It is important to note that analysis of stimulatory and inhibitory signals in u/dhESCs is relevant mainly for direct but not for indirect allore cognition, since the latter does not depend on antigen presentation by transplanted cells but rather the antigens are processed and presented by host’s APCs.

4. T-cell response against u/dhESCs measured by functional assays

From analyzing the expression of alloantigens and immune-modulating molecules in u/dhESCs, a complex picture takes shape; hESCs express MHC-I alloantigens which can be considerably elevated by IFNs and they rarely express co-stimulatory and MHC-II molecules. However, these data are not sufficient to draw solid conclusions about the immunogenicity of differentiated hESCs. Overcoming this difficulty requires functional assays for alloimmunity against preparations of differentiated hESCs. Classical test for alloimmunity is the mixed leukocyte reaction (MLR) in which leukocytes from two individuals are mixed together after inactivation of donor’s cells, and host’s leukocyte cytotoxicity serves as a measure for allorejection. The difficulty in adapting this assay to hESC derivatives is that most of these cells do not express co-stimulatory signals unless an enrichment protocol for APCs is used. In fact when T cells receive only MHC stimulus without co-stimulation they are likely to become anergic (Walker and Abbas, 2002).
To bypass the need for co-stimulation in MLR one could stimulate the recipient’s T cells by lymphocytes expressing the same HLA alleles as the hESC line in question. Using this approach, it was shown that IFN-stimulated u/dhESCs are rejected by T cells following incubation with the sensitized T cells (Drukker et al., 2006). These data imply that T cells may efficiently reject transplanted derivatives of hESCs if sufficient stimulation is provided. It is important to note that purified populations of therapeutic cells should be examined using similar assays in order to evaluate the expected intensity of the immune response against them. In addition, these assays are largely biased towards determining the potency of direct alloresponses, hence, other experimental procedures should be developed in order to further evaluate the potential of indirect alloresponses.

The most reliable assays for post-transplantation immunity are those performed in vivo. The difficulty with the development of animal models for tests of hESC-immunity is that they should carry functional human immune cells and provide the necessary environments for human immune responses. Together with colleagues, we have previously used the mouse trimera model (Reisner and Dagan, 1998) to test the human alloresponse against u/dhESCs (Drukker et al., 2006). In short, preparation of this model starts by adoptive transfer of bone marrow from severe combined immunodeficient (SCID) mice into normal irradiated mouse recipients. Then mice are infused with human peripheral blood mononuclear cells (PBMCs) that serve as the human immune components (Dekel et al., 2003; Drukker, 2006). Later, human leukocyte alloresponse against transplanted human cells and tissues can be evaluated. Using this model we observed that undifferentiated hESCs developed into teratomas and were not significantly rejected over the course of one month. Furthermore, transplantation of EBs, advanced teratoma fragments and a teratoma-derived cell line were also not rejected in this model. On the other hand, human skin grafts and transplanted human cell lines were showing clear signs of rejection indicating potent humanized immune response by the trimera model (Drukker et al., 2006). These data indicate that hESCs and their early derivatives are functionally less immunogenic than adult tissues.

There is considerable evidences to support the idea that hESCs like other embryonic cells are immunologically immature: the cells express relatively low levels of MHC molecules unless stimulated by IFNs and do not present co-stimulatory signals (see Figure 1D). Similarly, it has been shown recently that various fetal organs are resistant to immune rejection since they have low immune stimulatory potential (Dekel et al., 2003). Another factor that could influence immune evasion is the contribution of the grafted cells to neo-vascularization. As endothelial cells can also directly present MHC and co-stimulatory signals to the host T cells (Kreisel et al., 2002), their presence in the graft might have a negative effect on graft survival (Libby and Pober, 2001). At this point it is unclear to which extent hESC-derived cells give rise to endothelial cells following transplantation and additional experiments are warranted to examine the influence of these cells on transplant survival.

5. Interaction of natural killer cells with hESCs

Natural killer (NK) cells are cytotoxic lymphocytes possessing innate immune activities and can also affect adaptive immune responses. NK-cell mediated cell lysis is regulated by the balance of stimulatory and inhibitory signals that they recognize on target cells (Raulet, 2006). The prominent NK-cell inhibitory ligands include members of the MHC-I family as well as the non-classical human leukocyte antigens (HLA)-E and –G (see Figure 1C). As NK cells lyse cells that lack MHC-I expression, it is possible that u/dhESCs which express low level of MHC-I and HLA-E molecules would be targeted. However, when tested in vitro, activated human NK cells did not effectively lyse u/dhESCs (Drukker et al., 2002). Similarly, mouse NK cells showed no response towards u/dhESCs in vivo (Drukker et al., 2006). Thus far, no known NK-cell suppressive signal such as HLA-G molecules that protect embryonic trophoblasts from maternal NK cells (Kovats et al., 1990), have been shown to contribute to inhibition of NK cells by hESCs. Another explanation for the resistance of hESCs to lysis by NK cells may be due to lack of stimulatory signals for NK cells (see Figure 1C). Although these signals are still not well-defined, recombinant proteins of soluble NK-cell activating receptors and IgGs can be used to evaluate the expression of activating ligands on target cells. Indeed, it was demonstrated that expression of stimulating molecules for NK-cell activating receptors is very low or below detection level on u/dhESCs (see Table 1; Drukker et al., 2002). These data suggest that undifferentiated hESCs are protected from NK cells by virtue of not expressing lysis signals (see Figure 1D). It is also possible that the low expression of MHC-I is sufficient for protection and/or they express alternative inhibitory signals.

While human NK cell-response against hESC-derived teratomas seems to be minimal (Drukker et al., 2006), mouse NK cells were shown to reject mouse ESC-derived hematopoietic progenitors in vivo. Rideout et al., reported that these progenitors engrafted in mice lacking T, B and NK cells but not in Rag2/-/- mice that lack only T and B cells (Rideout et al., 2002). They also demonstrated that an antibody-mediated depletion of NK cells in Rag2-null
mice enhances hematopoietic progenitor engraftment. Since embryonic hematopoietic progenitors express low level of MHC-II molecules compared to adult hematopoietic progenitors (Huang and Auerbach, 1993), it is generally thought that NK cells lyse the embryonic cells as they are not sufficiently repressed. These data raise the possibility that in some cases MHC matched hESC-derivatives may be at risk of rejection by the patient’s NK cells. Further studies are required to determine whether NK-cell response will be a significant obstacle for engraftment of hESC-derivatives.

6. Generation of patient-specific isogenic hESC lines

Although hESCs derivatives seem to have reduced capacity to stimulate alloimmune responses, they are not considered as immune-privileged. Various approaches have been suggested to help alleviate the risk of rejection (Bradley et al., 2002; Drukker and Benvenisty, 2004), including genetic manipulation of MHC expression in the cells, induction of hematopoietic tolerance towards the cells and personalized production of isogenic cell lines. Of all these approaches, the last couple of years have been highly prolific for the latter – the production of patient specific pluripotent cell lines. There are currently three proposed techniques for derivation of such cell lines (summarized in Table 2):

1. Somatic cell nuclear transfer (SCNT): it has been shown extensively in mice that ESCs could be derived following SCNT into enucleated oocytes that are allowed to develop to blastocyst stage (Hochedlinger and Jaenisch, 2003). Similarly, it was shown recently that ESCs could be derived following SCNT into zygotes that had their chromosomes removed (Egli et al., 2007). It has been proposed that differentiated tissues from such “tailor-made” cell lines would not be rejected by the immune system of the nucleus-donor since they are genetically identical, except for mitochondrial DNA (mtDNA). Even though there is still no solid proof for the isolation of reprogrammed hESCs from cloned embryos, it is likely that overcoming several technical difficulties would allow the derivation of such cells in humans too.

2. Parthenogenesis: it has been shown for several mammalian species that activation of metaphase oocytes by chemical reagents can initiate development into blastocyst stage diploid parthenogenetic embryos. Protocols for ESC-derivation from such pseudo-zygotes were developed for mice (Allen et al., 1994) and recently human embryos. It has been shown by Revazova et al. that about half of the human embryos that were chemically activated, proceeded to the blastocyst stage and six parthenogenetic cell lines were derived. Importantly, it has been shown that the parthenogenetic cell lines have multi-lineage differentiation potential in vivo and in vitro (Revazova et al., 2007).

3. Induction of pluripotent stem (iPS) cells from somatic cells by genetic factors: the seminal experiment of producing cell lines that highly resemble mouse ESCs by transferring four transcription factors – Oct3/4, Klf4,
Sox2 and c-Myc into somatic cells has opened a new avenue for “tailored” production of pluripotent cell lines (Takahashi and Yamanaka, 2006). In addition, it has been demonstrated that the same factors or similar combinations can be used for derivation of human iPS cells from somatic cells (Park et al., 2008; Takahashi et al., 2007; Yu et al., 2007). Application of this technique for clinical settings still requires in-depth analysis of the multi-lineage potential of iPS cells and introduction of non-oncogenic reprogramming factors without retroviral integration into the genome.

There are different immunological considerations for clinical use of cell lines generated by each of these techniques. One immunological issue, which is unique to SCNT, is potential immune response directed against mitochondrial histocompatibility antigens. It is known in mice and rats that certain amino acid substitutions in mitochondrial proteins can lead to the generation of alloreactive T cells (Loveland et al., 1990). Therefore, it is possible that some mitochondrial protein polymorphisms may become antigenic and provoke chronic immune responses following transplantation of cells that were prepared by SCNT. However, the relatively small number of mitochondrial encoded proteins and the small number of mitochondrial alleles suggests that the risk of immune response towards donor-derived mitochondrial mHAgS would be considerably lower than against mismatched genomes. In accordance with this hypothesis, it has been shown in cows that transplantation of organs derived from cloned embryos to the nuclear donor did not lead to immune response even though they have different mitochondrial haplotypes (Lanza et al., 2002).

Parthenogenetic hESC lines are not expected to stimulate any adaptive immune response since they are genetically identical with the oocyte donor. However, NK cells may actually respond against parthenogenetic hESC lines that lack one set of MHC-I encoding genes. It was demonstrated in mice that heterozygotic bone marrow cells containing two MHC-I haplotypes, rather than one, engraft better in isogenic hosts that have two distinct MHC-I haplotypes (Hoglund et al., 1997). Therefore, a potential concern for transplantation of cells derived from MHC-homozygous parthenogenetic hESC lines (Revazova et al., 2008) is the risk of rejection by NK cells due to reduced diversity of MHC alleles.

The extent of MHC homozygosity in parthenogenetic hESC lines depends on oocyte activation stage, metaphase-I or -II (M-I and M-II, respectively) and on recombination events (Kim et al., 2007). Activation of M-I oocytes gives rise to cell lines that contain the two maternal chromosome homologs whereas M-II activated oocytes give rise to cell lines that have only half of the maternal homologs. This still does not rule out heterozygosity of multiple loci in M-II activated oocytes due to recombination between the homologs. In fact, it was found by Revazova et al., (Revazova et al., 2007) that all parthenogenetic hESC lines that they have derived from M-II oocytes contained full heterozygosity in the MHC region. It should be noted that parthenogenetic hESC lines were probably also derived by Hwang’s group in South Korea, although, the team reported derivation of SCNT-derived hESC lines (Hwang et al., 2004). Genome-wide analysis of one of the “cloned” hESC cell lines showed homozygosity in centromeric regions but extensive heterozygosity in telomeric regions, indicating that it is a parthenogenote (Kim et al., 2007).

Possible benefit for derivation of parthenogenetic hESC lines that contain homozygosity in the MHC region (Revazova et al., 2008) is their possible use in individuals that are genetically related to the oocyte donor. For example, there is 50% chance that a cell line that is derived from a patient will be histocompatible to any of her children if it has two identical MHC alleles. Moreover, if a sizeable depository of MHC homozygous parthenogenetic hESC lines were to be generated, it may serve as a MHC-matching cell lines bank (Rubinstein, 2001). This holds true since MHC homozygous hESC cell lines would have a much higher matching frequency than heterozygous cell lines. The extent of NK-cell response against such transplants remains unclear and should be investigated. Also, transplantation of differentiated parthenogenetic hESC to genetically non-identical donors may involve the risk of inducing immune response against mHAgS and mitochondrial antigens.

The only approach that currently holds the promise to generate absolute isogenic cell lines is reprogramming somatic cells into iPS cells using defined genetic factors. Still, it is important to note that adaptive immune responses may occur against embryonic mis-expressed antigens and that NK-cell response is a possible risk for transplanted embryonic cells that express low levels of MHC molecules. Therefore, this aspect should be further examined and strategies to derive iPS cells without stable integration of retroviruses and oncogenes should be developed. If differentiated iPS cells would prove to be safe for transplantations, it is very likely that this approach will become the primary source of isogenic cell lines for clinical use.
7. Conclusions

Decades of research devoted to tissue rejection have uncovered many of the molecules and cell types that mediate the immune processes following allotransplantation. Through these investigations it has become clear that almost every aspect of adaptive and innate immunity may be involved in rejection. The isolation of hESCs and their differentiation capacities has created a novel pathway for generating cells with potential therapeutic relevance on an unprecedented scale. However, it is still premature to predict the exact immunological outcomes after implantation of differentiated hESCs before production of therapeutic grade cells will be achieved. The emerging picture from the existing data show that hESCs and their early-differentiated derivatives are immunologically immature; they express lower than somatic levels of MHC-I and do not express MHC-II and co-stimulatory molecules unless directed to differentiate to certain hematopoietic lineages. Thus far, functional in vivo studies have indicated that their limited co-stimulatory potential reduces their risk for direct rejection by cytotoxic T cells, though MHC-I levels are sufficient for T cell response by activated cells. To further reduce the potential for direct allorecognition a potential approach may be to deplete APCs from transplanted cell preparations by specific antibodies.

In contrast to direct allorecognition, indirect alloresponse in most cases does not require expression of co-stimulatory molecules by the transplanted cells and virtually any polymorphic protein can induce immune response. Therefore, it is expected that indirect chronic responses would develop against MHC molecules and mHAgs following transplantation of allogeneic differentiated hESCs. Still, predicting the severity and rate of rejection is particularly hard since multiple factors, including genetic disparities, tissue trauma during surgery and sites of transplantation can significantly influence inflammation-promoting processes which have a primary role in initiation of immune rejection. As it turns out that endothelial cells possess at least some of the stimulating functions of APCs they may also play an important role in development of alloimmune responses.

Finally, new key developments in derivation of partially or fully isogenic pluripotent human cell lines may now enable transplantation of their derivatives with only very low risk of immune rejection. Induction of pluripotency in somatic cells is of particular importance since it does not require the use of human oocytes and zygotes and the resulting cell lines are fully compatible with the donor. It is important to note that if pluripotent cell line would be derived following SCNT into oocytes and zygotes or parthenogenesis, the reprogrammed cell lines may carry allogeneic mitochondrial antigens and parthenogenetic cells might be lysed by NK cells. Regardless of the hESC source, detailed examination of their recognition by various immune cells is required since in vitro differentiation may lead to reduced levels of MHC expression and immature cells may express embryonic antigens that are not familiar to the adult immune system.

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9. References


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