Semin Immunopathol (2011) 33:525–534
DOI 10.1007/s00281-011-0262-z

REVIEW

How to cross immunogenetic hurdles to human embryonic stem cell transplantation

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Received: 6 January 2011 / Accepted: 15 March 2011 / Published online: 2 April 2011

Abstract Implantation of human embryonic stem cells (hES), derived progenitors or mature cells derived from hES has great therapeutic potential for many diseases. If hES would come from genetically unrelated individuals, it would be probably rejected by the immune system of the recipient. Blood groups, MHC and minor antigens are the immunogenetic hurdles that have to be crossed for successful transplantation. Autologous transplantation with adult stem cells would be the best approach but several elements argue against this option. Classical immunosuppression, depleting antibody, induction of tolerance and stem cell banking are alternative methods that could be proposed to limit the risk of rejection.

Keywords Embryonic stem transplantation · MHC · Minor antigen · Rejection · Immunosuppression

Abbreviations

hES	Human embryonic stem cells
NK	Natural killer
MHC	Major histocompatibility complex class
HLA	Human leukocyte antigen
mHC	Minor histocompatibility antigen
GvHD	Graft-versus-host disease

This article is published as part of the Special Issue on Immunopathology of Pluripotent Stem Cell Transplantation-[33:6].

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Introduction

Although human embryonic stem cells (hES) have been studied for more than 50 years [1, 2], it is only recently that they became a sensitive topic. hES cells are pluripotent stem cells, isolated from the inner cell mass of human blastocysts. The two salient characteristics of ES cells are that they are undifferentiated and that they are capable of self renewal.

HES cells are very convenient for clinical purposes because they can be derived into different progenitor cells types, being an inexhaustible source of mature cells for organs and tissues.

Implantation of progenitor or mature cells derived from hES has great therapeutic potential for many diseases and can be viewed as the "holy grail" of transplantation and regenerative medicine.

The origin of hES is of great importance since transplanting a patient with cells from a genetically unrelated donor (allogenic transplantation) will trigger a detrimental immune response. Seminal publications strongly suggest that hES cells possess immune-privileged properties [3]. Thus, transplantation of hES into organs like the brain, which is also considered an immune-privileged site, was viewed as a typical example of a potentially curative treatment of neurodegenerative diseases without incurring the risk of an unwanted immune reaction [4]. More recent works imply that the risk of rejection by the immune system of allogenic hES or their progenitor cells warrants serious thought. T lymphocytes and natural killer (NK) cells are able to target hES and their progenitor cells, and immunosuppressive treatments have to be included in protocols of transplantation. Interestingly, recent publications strongly suggest that hES cells possess mechanisms that protect them from the immune system. Indeed, hES

cells seem to be able to escape the destruction mediated by T or NK cells by means of a serine protease inhibitor [5].

In this review, we will discuss extensively the immune status of hES cells and we will describe the immune hurdles to the transplantation of hES cells and derived progenitors. Finally, we will focus on several approaches to overcome the barriers of genetically unrelated individual. As a matter of fact, hES cells, their progenitors or fully mature cells possess different properties. This could be a crucial factor in determining the risks and the chances of success of hES cells transplantation.

Immune barriers to hES transplantation

The problems arising from transplantation—whether of tissue, organs or stem cells—between two genetically unrelated individuals (allogenic) are similar. If the immune response is not suppressed, the body will definitely reject the graft, i.e. in this case the ES cells. Rejection occurs because of the presence of multiple different antigens between the donor and the recipient that activate the innate and adaptive immune systems. Three "families" of transplant antigens are known to play an important part in inducing solid organ rejection:

- The human ABO blood-group antigens
- The major histocompatibility complex (MHC)
- The minor histocompatibility antigens.

The human ABO blood-group antigens

ABO antigens are the result of structural polymorphisms in carbohydrate residues bound to the cell surface. Nearly all cells, but particularly erythrocytes, express these ABO antigens. Organ transplantation between an ABO incompatible donor and recipient cause hyperacute rejection, mediated by preformed specific antibodies to ABO antigens that activate the complement cascade [6]. ABO incompatibility leads to hyperacute rejection in vascular organ transplantation, but it does not cause problems in hematopoietic stem cell transplantation. This is due to the fact that in hematopoietic stem cell transplantation, the recipient blood group will be replaced by the donor blood group. The immune system reconstituted after the transplantation will be tolerant to recipient ABO antigens expressed by the non hematopoietic cells of the recipient. As far as we know, only one study investigated the expression of A and B antigens in hES cells, demonstrating the expression of AB antigens according to the ABO genotype embryonic stem cells [7]. Several A genotype hES cell lines stained positive with anti-B antibody. After derivation of hES into hepatocyte- and cardiomyocyte-like cells at different stages of maturation, originating from a B blood-group human embryonic stem cells (hESC) line, showed that hepatocyte-like cells expressed B antigens whereas cardiomyocyte-like cells were negative [7]. In the human system, ABO incompatibility between donor ES cells and the recipient that would lead to hyperacute rejection, remains to be proven, but a possible solution might be to use of ES cells from a type O blood donor (universal donor).

The MHC

The human leukocyte antigen (HLA) gene is the most highly polymorphic gene in the human genome. Two major gene products are encoded by the HLA, the major histocompatibility complex class I (MHC-I), and the major histocompatibility complex class II (MHC-II). The classical MHC-I molecules (HLA-A, HLA-B and HLA-C) are expressed by nearly all nucleated cells, and the classical MHC-II molecules (HLA-DR, HLA-DQ and HLA-DP) are expressed constitutively by dendritic cells, macrophages or B cells but can be induced by cytokines like interferon gamma (IFN-γ) on nearly every cell type. The role of MHC class I and II expressed by dendritic cells is to present antigenic peptides to specific T cells in the lymph nodes. Specific T cells will migrate to the tissue and target cells that present the same antigen. If the antigen is from an unrelated individual or emanates from a microorganism, T cells will destroy it. MHC antigens are also strongly immunogenic, and thus the presentation of MHC from an MHC mismatched donor will activate the recipient's immune system. Therefore, the expression of MHC at the cell surface is the greatest immunological barrier to transplantation. In hematopoietic stem cell transplantation, incompatible MHC between donor and recipient is the source of either graft-versus-host disease (GvHD)—where graft T cells can react to recipient's HLA—or graft rejection, where the receiver's T cells attack the graft. The highest risk of GvHD or graft rejection is between HLA-mismatched individuals. In solid organ transplantation, the immune reaction is only direct into the graft direction, the immune system being on host origin only.

The question of MHC expression by ES cells is crucial and has been scrutinized by several groups. Drukker et al. [8] showed that ES cells express very low levels of MHC-I protein on their surface, and after differentiation in vitro or in vivo, the level of MHC-I expression increases only slightly. However, when ES cells are treated with IFN-γ, a marked induction of MHC-I can be observed. On the other hand, MHC-II is not expressed on ES cells, even when they are activated with IFN-γ. Another publication [9] reports that human pre-implantation embryos do not express MHC-I or MHC-II.

At least, two aspects would increase the immunogenicity of ES cells. Even if ES cells originally express MHC-I in

small amounts, this would naturally increase after their normal differentiation into a different lineage, at least as far as MHC-I is concerned. Moreover, IFN-γ is often present during infection or inflammation and this would also increase the level of MHC-I, but the risk of rejection by allogenic ES cells has to be addressed.

In addition to the classical MHC protein, the role of non-classical MHC protein like HLA-G and E that can bind the receptors of NK cells KIR2DL4 and NKG2A, respectively, of NK cells, remain to be addressed in the context of the alloimmune response.

The minor histocompatibility antigens

Without a doubt, ES cells express minor histocompatibility antigen (mHC). These antigens are mainly derived from mitochondrial DNA (mDNA) and from Y-chromosome genes (H-Y antigens). But all polymorphic proteins (different between the recipient and the ES cells) are potentially immunogenic and should be considered minor antigens. They are presented as peptides by the donor's antigen-presenting cells (APC). Y-chromosome gene products are responsible for immunological reactions in transplants between males and females, female T cells responding to H-Y antigens. The immune reactivity of mHC is less strong than MHC incompatibility but it can induce allograft rejection and should not be underestimated [10].

Allorecognition pathway

The immunological mechanism that causes rejection and involves recipient $CD4^+$ T cells consists of two distinct pathways; the direct (Fig. 1a) and the indirect (Fig. 1b) allorecognition pathway. In the direct pathway, a graft is transplanted with APC, or with APC- precursors that will differentiate into APC after transplantation. These transplanted APC migrate to the nearest lymph node where they meet recipient T cells. These recipient $CD4^+$ T cells, that express T cell receptors, bind to the MHC-II–peptide complex expressed by the donor APC, which triggers an immune reaction. The indirect pathway is also triggered when the recipient's APC express the MHC-II receptor with donor antigens such as dying or apoptotic–necrotic transplanted cells. The recipient's APC activates the recipient's T cells in the same manner as in the case of the direct pathway, through co-stimulatory molecules such as CD40, CD80 or CD86. The immune reaction with transplanted ES cells would probably involve mainly the indirect pathway. ES cells have no APC properties but can be derived into APC. However, APC could be derived from ES cells, in such situation APC would originate from the donor and could drive the direct pathway (Fig. 1b) [11].

In addition to T cells, NK could also be part of the alloreaction to hES cells. NK cells are a sub-population of cells from the innate immune system and express different activating and inhibitory receptors (Fig. 2). According to the "missing-self" hypothesis [12], NK cell receptors bind to self-MHC-I and inhibit NK cell activity, whereas NK cell cytolytic activity is directed against cells that have lost self-MHC-I expression. NK cell receptors need no specific rearrangement like T cells do, they are fully functional and their repertoire of receptors differs from individual to individual. The absence of MHC class I in hES cells make them potential targets for NK cells.

Immune response to allotransplanted ES cells

The amount of information available on the immune properties of ES cells is not extensive. Before using these cells in a clinical trial, their immunogenicity must be well understood. Drukker et al. [13] answered part of this question by using two different animal models with hES cells. The first model, based on an immunocompetent mouse, was used to clarify the mechanism underlying hES cell rejection. The purpose of the second one, a mouse which carried the human peripheral blood lymphocytes, was to test the immune response to differentiated and undifferentiated hES cells. In the first model, after 1 month of experiments, the injected hES cells failed to develop into teratomas, contrary to hES cells in NOD/SCID mice that derived into teratomas. Drukker et al. demonstrated that the rejection mechanism was mediated by T cells. By using humanized mice, hES cells were not rejected and developed normally into teratomas, in contrast to human adult skin grafts, which were infiltrated by leukocytes. Some investigators suggest that if these ES cells escaped rejection, it was probably due to their immunological immaturity, characterized by the non-expression of MHC-II and co-stimulatory molecules and the weak expression of MHC-I. Moreover, they showed by means of a DNA microarray that half of the immune genes expressed in the hematopoietic cell line, were not up-regulated on human ES cells. This could justify reducing immunosuppressive drugs in ES cell transplantation. It has to be emphasized, however, that those experiments were performed over a short period of time whereas conducting them on a long-term basis might reveal if the weak expression of MHC-I on ES cells would increase after their derivation and maturation.

Several studies show that ES cells can escape immune recognition. Human ES cells have been shown to survive for many weeks after transplantation in rats [14]. It should be noted that all these studies were carried out during immunosuppressive treatment.

Other groups have described that murine ES cells transplanted in allogenic recipients triggered the immune

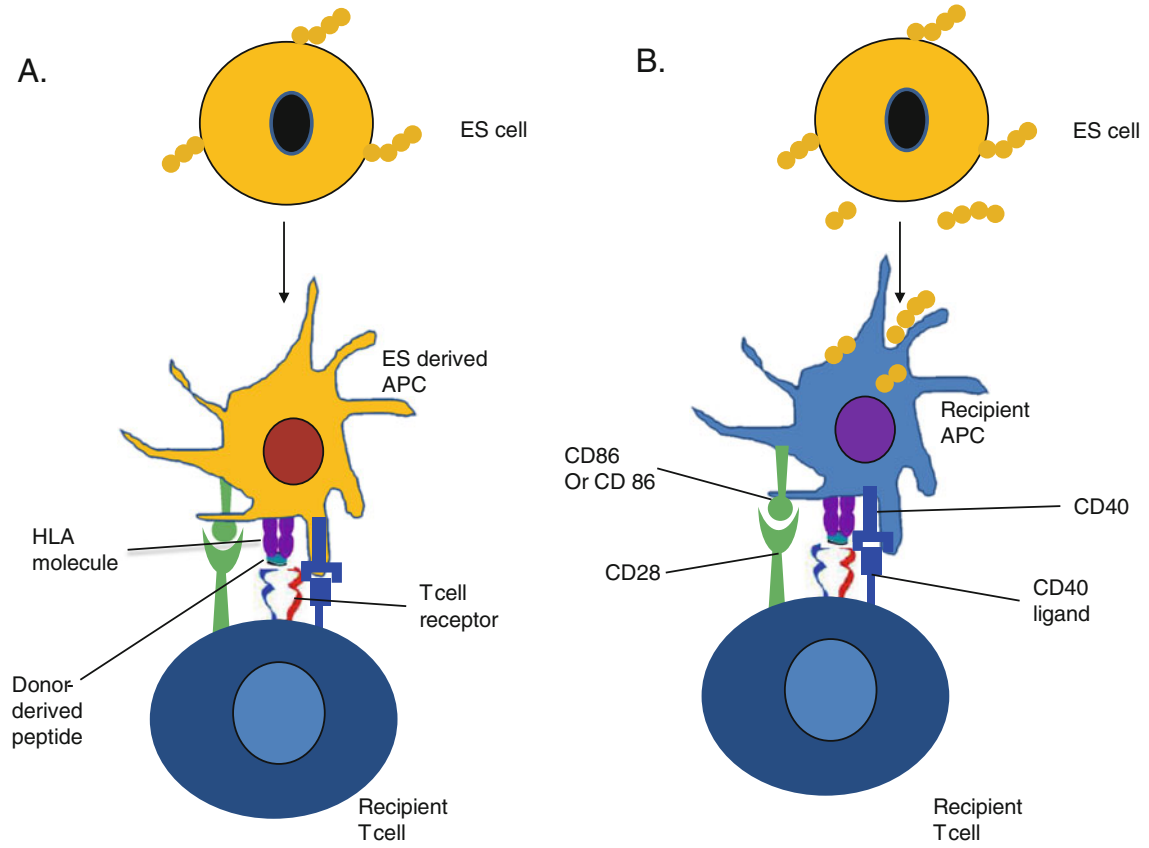

Fig. 1 Illustration of direct (*left*) and indirect (*right*) rejection mechanism mediated by antigen-presenting cells (*APC*). The APC leading to direct pathway presentation would be derived from ES

response and were rejected. Thus, Swijnenburg et al. [15] demonstrated this very clearly in a non-invasive experiment by injecting hES cells, transduced with a green fluorescent protein, into the leg muscles of mice with either normal or compromised immune systems. They screened the hES cells by visualizing the fluorescence under a microscope and showed that in the immunocompetent mice hES cells died after a few days, whereas in the immunodeficient mice ES cells survived longer. When transplantation was repeated, hES cell death was accelerated, which highlighted

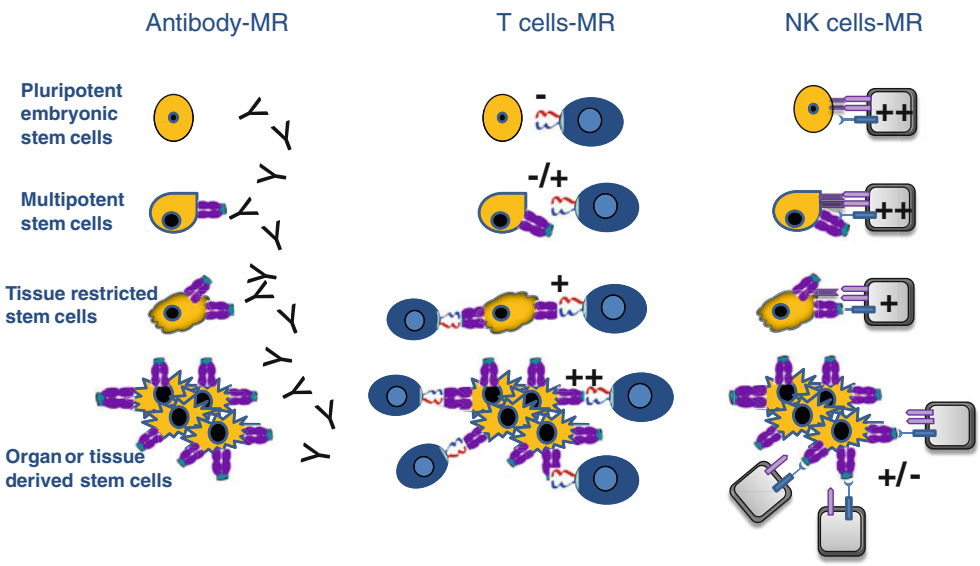

Fig. 2 Potential immune reactivity mediates by ABO or anti-HLA antibodies (*Ab-mediated rejection*), T cells (*T cells-MR*) or natural killer cells (*NK-MR*) according to the state of stem cell differentiation

the memory immune response mediated by CD4 T cells. Finally, when normal mice were treated with immunosuppressive drugs (tacrolimus or sirolimus), hES cells survived for a longer period.

The fact that MHC-I expresses so few ES cells could be a real disadvantage when faced with NK cells. By assessing the expression of MHC-I on ES cells Drukker et al. [8] intended to find out if they would be recognized and lysed by NK cells. Their standard cytotoxic assay revealed that the level of ES cells killed was very low compared with that of NK-sensitive cell lines. In the presence of IFN-γ, a slight increase in the killing activity was observed. This could mean that in the presence of ES cells, the killing mechanism does not depend on MHC-I. Considering that NK cells were not previously activated by pro-inflammatory cytokines like IL-2 or IL-15, the response was less marked than would be expected in the case of an inflammatory condition.

Interestingly, it has been demonstrated that ES cells have "a sort of" individual system of defense to avoid immune rejection by T cells and especially by NK cells. ES cells adopt 2 mechanisms to escape lysis by effector cells. The first is composed of two ligands, CD95L and FasL, which induce apoptosis of the cells expressing CD95 or Fas. This may explain why there are so few T cells at the site where ES cells are implanted. The second mechanism involved in the survival of ES cells is the expression of high levels of cathepsin B and a serine protease inhibitor (serpin), SPI-9 [16]. SPI-9 allows the destruction of granzym-B, a serine protease released by cytotoxic T cells and NK cells. Interestingly, when SPI-9 is knocked-down by siRNA, ES cells are easily killed by target cells. Of note, several malignant tumors express SPI-9, which allows them to elude the immune system [17].

The expression of serpin seems absolutely crucial for ES cells. Adult stem cells are the only source of cells that regenerate after injury or normal homeostasis. If these adult stem cells are infected by pathogens and therefore killed by cytotoxic T cells or NK cells, the organism's survival is at risk. A parallel can be drawn from a lethal dose of irradiation that destroys the bone marrow and, as a consequence, precludes the reconstitution of the hematopoietic cell lineage.

Finally, serpin is most certainly responsible for the non-rejection when the blastocyst encounters the mother's uterine epithelium. In experiments involving mice models [18], authors showed that after 4 to 5 days post-conception, SPI-6 (mouse homologue of human SPI-9) was present in the outer cell layers of the embryo, whereas elsewhere in the embryo no SPI-6 was expressed. Later (12 days post-conception) SPI-6 existed exclusively in the placenta. Authors suggest that it was due to the expression of SPI-6 at the site of contact between embryo and mother that this half-allogenic embryo (50% of the genes originated from the father) was tolerated by the mother's immune system.

To overcome the immune barriers

At first glance, ES cells offer a great advantage compared with other tissues. As they express HLA-I molecules in very small amounts, their immunogenicity should be accordingly low. However, due to the risk of teratoma and also some uncertainty with regards to the in vivo differentiation into specific lineages and tissue, it would be more efficient to transplant progenitor or mature cells rather than hES cells. The consequence would be an increased risk of rejection [19]. This type of transplantation could be viewed as a classical procedure in tissue or organ transplantation with similar immunological risks of rejection.

In this part, we will discuss several ways to minimize the allogenic reaction between donor and host, which is achieved by means of classical immunosuppressive therapy and the induction of tolerance in order to overcome the immune barrier to stem cell transplantation.

Immunosuppressive therapy

To prevent rejection efficiently, the use of different immunosuppressive drugs (ID), which possess diverse mode of action, is recommended. While in the past acute rejection of transplanted organs or tissue was common, nowadays, thanks to these drugs, graft survival is steadily on the increase.

The aim of ID is to impede four main cellular mechanisms and to achieve the following effects; (a) blockade of T cell proliferation (by anti-metabolites like azathioprine or mycophenolate mofetil); (b) reduction in inflammation (by steroids); (c) inhibition of cytokine production (by calcineurin inhibitors like ciclosporine or tacrolimus); (d) finally, depletion of macrophages/APC, T cells and/or B cells (by monoclonal antibodies, like rabbit anti-thymocyte globulin (rATG), rituximab or alemtuzumab).

Steroids or corticosteroids act as glucocorticoid receptor agonists, which target transcription factors such as NF-κB. This therapy is used to reduce inflammation and to induce T cell apoptosis. Steroids are still very important immunosuppressors, especially in the first month after transplantation. Then, during the first year after transplantation, steroids are reduced due to their side effects such as bone loss or gastro-intestinal problems [20].

In 1968, azathioprine [21] was the first immunosuppressive drug to be used on a regular basis in organ transplantation. It acts by releasing a compound, 6-mercaptopurine, which interacts with DNA synthesis. Azathioprine has however been replaced progressively by mycophenolate mofetil, which inhibits inosine-monophosphate deshydroge-

nase (important for the synthesis of purine and selectively used by T and B cells).

Cyclosporine (CsA) and tacrolimus (TaC) [22] are two compounds that interact with calcineurin. Calcineurin is a protein, which binds to a transcription factor, NFAT (nuclear factor of activated T cells). This complex increases the expression of IL-2 and stimulates the development and the differentiation of T cells. Depending on the patient and the side effects, either CsA or TaC is used but never both together. These two ID have been proving their efficacy for several years [20]. Sirolimus, or its derivative everolimus, blocks the intracellular protein mammalian target of rapamycin (mTOR), which activates T cells by signaling the IL-2 receptor. By binding to mTOR, sirolimus blocks the IL-2 pathway and inhibits T cell proliferation.

The role of immunossuppressive drugs is to block the immune system but unexpected events have to be prevented before using ID. NPC from a fetal origin or from hESC have the potential to differentiate into mature neurons after transplantation into the brain, opening the possibility of regenerative cell therapy for neurodegenerative disorders like Parkinson's disease. To prevent the T and NK cells immune response several ID have been used in vitro to test their effect on culture NPC. We have demonstrated that cyclosporine and dexamethasone strongly inhibit the terminal differentiation of NPC into mature neurons [23].

Interestingly, this effect could be species dependent or dependant of the source of stem cells. Indeed, in a mouse model of NPC transplantation derived from mice neural stem transplantation (and not from embryonic stem cell), administration of cyclosporine A to adult animals increased the numbers of NPCs within the neurogenic niche lining the lateral ventricles. These findings suggest that cyclosporine A has direct effects on NPCs both in vitro and in vivo, making it a promising candidate molecule for developing clinically relevant strategies to stimulate NPCs for brain repair [24].

Therefore ID should be carefully considered in the context of allogeneic transplantation of human hES if steps of differentiation are request after transplantation.

The good regimen should combine the ability to inhibit the T and the NK cells response without interfering with the terminal differentiation into mature cells.

Depleting drugs and antibodies

Allograft rejection is a process involving mainly T cells, and the specific depletion of recipient's T lymphocytes has been actively developed using mostly mono- and polyclonal antibodies. Recombinant polyclonal rATG is at present one of the most common drugs used to deplete T cells. These polyclonal antibodies are obtained by immunizing rabbits with human lymphoid cells. rATG rapidly induces lymphocytopenia by different processes such as opsonization, apoptosis or complement-dependent lysis. rATG does not only contain T cell antigens such as CD3, CD4 and CD8, but also B cells antigens such as CD5, CD19 and CD20. In different studies on animal models it was shown that rATG increases regulatory T cell subsets (T-regs) and NK cells. T-regs play an important role in the regulation of the activating T cells and prevent autoimmune disease and GvHD [20].

A monoclonal antibody targeting anti-CD52 (alemtuzumab) expressed by T cells, NK cells and monocytes is also very efficient in depleting immune cells involved in the rejection mechanism [25]. Last but not least, the binding of the alpha chain of CD 25 by a chimeric monoclonal antibody (basiliximab) blocks the IL-2 transduction pathway and contributes to the prevention of rejection. As far as we know, depleting antibody has never tested in model of ES transplantation [26].

Induction of immune tolerance

Immune tolerance prevails when the immune system does not react to antigens. Technically speaking, three types of tolerance exist: (a) central tolerance or thymic tolerance which occurs during lymphocyte maturation and takes place in the thymus in the case of T cells and in the bone marrow for B cells. Autoreactive T and B cells are eliminated during these processes. (b) Peripheral tolerance, or non-thymic tolerance, where mature lymphocytes move into the periphery and do not react to foreign antigens. (c) Acquired tolerance, an active process during which regulatory T cells block the activated T cells [11].

The induction of immune tolerance is the "holy grail" of transplants specialists that consist in the blockade of the alloimmune response to the graft without inhibiting the immune response to microorganisms. Due to their weak immunogenicity the use of ES cells has also been propose to induce tolerance.

In 2002, Fändrich et al. [27] disclosed the first data on tolerance induction using donor-derived embryonic stem cells in a rat model. Instead of irradiating the host to defuse the immune system, they injected rat embryonic stem cells (rESC) isolated from blastocysts. Subsequently, these cells were found in the liver, spleen, mesenteric lymph node and thymus, which highlight the migration ability of these cells. Moreover, these rESC develop into B cells and monocytes, but no T or NK cells were found. rESC tolerated a cardiac graft from the same rat strain as their own. So probably, undifferentiated ES cells would not be recognized by T cells and treatment based on ES cells is possible, if the thymus is fully functional. But the risk of teratomas is too high in humans if undifferentiated ES cells are transplanted.

Immunomodulatory properties of ES have to be considered in the context of transplantation but currently it is mainly the mesenchymal cell population that has the greatest potential of regulation with regards to T and NK alloimmune response [28]. Dazzi reviews this topic in another article of this issue.

Finally, the role of regulatory T cells coming from the recipient or derived from embryonic stem cells of donor origin to induce tolerance need to explored in the context of embryonic stem cells transplantation.

Mixed chimerism

Mixed chimerism is a state where donor's and recipient's hematopoietic cells co-exist in the same organism [29]. This situation has been observed after hematopoietic cell transplantation. T cells originate from hematopoietic progenitors. They respond to antigens in secondary lymphoid tissues such as lymph nodes or the spleen. But in order to respond to antigens, T cells need to learn to differentiate between self and non-self antigens. This recognition process takes place in the thymus. The thymus is divided into several distinct functional areas, the cortex, the cortico-medullary junction and the medulla. Progenitor T cells from the bone marrow migrate to the thymus through blood vessels at the cortico-medullary junction. Once in the thymus, they proliferate, differentiate and migrate to the cortex. In the cortex, the double negative CD4/CD8 thymocytes mature into double positive CD4/CD8 thymocytes and encounter cortical thymic epithelial cells, which express a peptide-MHC complex. The survival cells are those, which express functional T cell receptors that in turn bind to the peptide-MHC complex. After this first selection, a second selection takes place, where the double positive cells mature into single positive ones and return to the medulla, where they leave the thymus to join the periphery [30]. Knowing how the thymus works, one could imagine that allogenic reactivity might be avoided by mixing donor and recipient T cell progenitors. Chimeric recipients would have specific immunological tolerance to the antigen expressed by the donor, and they would not reject the allograft. This promising technique would make it possible to circumvent the immunological barrier that constitutes HLA [11]. Promising results have already been obtained in human kidney transplantation [31].

Autologous transplantation

To overcome the immune reaction and to achieve a perfect immunological match between an embryonic stem or progenitor cells of donor origin and the recipient, the use of induced pluripotent stem cells (iPS) cells would be a solution. iPS are obtain, by transducing four important genes, *Oct-4, c-Myc, Sox-2* and *Klf-4*, involved in the development of ES cells, into a somatic cell. The resulting iPS expresses the different characteristics of an ES cell [32–35]. Recent work suggests that *Oct-4* alone would be sufficient to induced pluripotency in adult neural stem cells [36].

However, new difficulties would arise. For example, in disease with a well-documented genetic defect, the mutated genes will be present in the iPS cells. This occurred in patients with spinal muscular atrophy [37], and iPS generated from fibroblasts of a sick child proliferated in culture while maintaining the genotype of the disease. One advantage of this phenomenon is that these iPS could be used as a tool to study in depth the genetic defect and develop new treatments, which would be much more relevant than animal models. On the other hand, it would be a pity if iPS cells produced a protein that is absent in the deficient host. The immune system of the host may identify this protein as being non-self and induce an immune reaction, which is not wanted. In addition, it has to be pointed out that several diseases, of which neurodegenerative diseases, have unknown causes. What would happen if iPS from these patients carried the defect? This will have to be clarified. As far as the derivation of iPS is concerned, it would probably be preferable in this context if the cells used for derivation originated from a genetically unrelated donor [4].

Finally, preparation of cells of iPS of clinical grade, suitable for transplantation into patients could be difficult to achieve for time- and cost-effective reasons. The derivation, maintenance and differentiation of iPS (like for hES) should be accomplished under xeno-free culture conditions using good manufacturing practice systems [38–41]. Guided-differentiation methods will have to be established, leading to homogeneous and reproducible cell populations that do not form teratomas or cause cancer [42].

Stem cell banking

To overcome the problem described above, inherent in the use of iPS for autologous transplantation, the characterization of hES or iPS cell lines has to be explored. Homozygous cell lines with frequent HLA haplotypes could theoretically minimize the risk of reaction. Several publications emanating from various countries specify the minimal number of cell lines that would cover a significant population with regards to their HLA typing [43–46]. However, these studies did not include high-resolution HLA typing, and thus the significance of their results in terms of rejection is limited. Coming from genetically unrelated donors, the presence of minor antigens would signify that even in the case of HLA compatibility as determined by high-resolution typing, the risk of rejection remains unless the patient's immune response is suppressed.

Thus, the last hurdle to overcome is to determine the level of immunosuppression required in each individual case depending on compatibility. However, work of the past decades in solid transplantation clearly indicates that it is almost impossible to know beforehand the correct dosage of immunosuppressive drugs that is required for a given patient.

Conclusions and perpectives

ES cells are isolated from the inner mass cells of blastocysts. After culture in specific media, these ES cells can be derived into various types of cells or tissues. Moreover, ES cells can renew themselves. One of the biggest controversies surrounding ES cells is the use of blastocysts, which are the result of an ovocyte fertilized by a spermatozoid. Once the ES cells are isolated from the inner mass cells and cultured, the residual blastocysts are destroyed, which gives rise to ethical issues. Consequently, other solutions need to be found that afford sufficient amounts of ES cells without the use of blastocysts. Nowadays, several techniques exist, the most recent of which consists of reprogramming somatic cells that generate iPS cells, which have the same properties as ES cells.

Several groups show that if ES cells are cultured in well-defined conditions, they are able to derive into other cells tissues such as neurons [47], cardiomyocytes [48] and hepatocytes [49], to name but a few, each of which possess the same specific properties as the original cells.

The fact that ES cells are able to renew themselves and are derived into other types of cells renders them very attractive for the replacement of injured or even destroyed tissues. In transplantation, cytotoxic T lymphocytes CTL and NK cells are the two main actors in the rejection process. However, since receivers need to be treated with ID in order to reduce the activities of T cells, only NK cells can be taken into consideration.

As ES cells express very low amounts of MHC-I or none at all, nor any MHC-II, they would be perfect targets for NK cells, which lyse MHC-I negative cells, according to the “missing-self” hypothesis. But it seems that this is not the case [8] when unstimulated NK cells are used.

An important issue, common to many publications, is the time factor. All the studies investigating the immunogenic response of ES cells to NK or T cells were performed over a short period of time. What would happen over time when ES cells were cultured and derived? Frenzel et al. [50] answered this question to some extent. In a mouse model they assessed the difference between undifferentiated ES cells and ES cells derived from cardiomyocytes (ESCM). Although both cell types express similar low levels of MHC-I, ESCM are neither recognized nor lysed by NK cells, but ES cells are both recognized and lysed by NK cells. The study showed that the difference lies in the expression of NKG2D ligands, which are markedly expressed by ES cells, whereas ESCM do not express these ligands. This demonstrates that after derivation ES cells may change their phenotype.

In order to prevent rejection of the ES cells, several methods for monitoring the immune response are available. Obviously, it is easier to culture ES cells in vitro than to monitor them in vivo. The culture of ES cells and their differentiation into progenitor cells make it possible to conduct several analyses, such as crossmatch or mixed-lymphocyte reaction, to test their immunogenicity. Cross-match assays reveal the presence of antibodies in the serum. The recipient's serum is incubated with ES cells, and by means of FACS technology the binding of antibodies to ABO, HLA or other antigens can be analyzed. A positive result indicates a high risk of rejection. NK or T cells can be analyzed as well by chromium release cytotoxic assay. ES cells are incubated with radioactive chromium and the functionality of the effectors cells is then estimated. The amount of radioactive chromium released by ES cells in the supernatant is indicative of NK or T cell activation.

The aim of this review is to highlight the immunogenicity of ES cells. Despite the fact that ES cells are undifferentiated cells with low or no expression of MHC-I and MHC-II, their implantation triggers an immune reaction. There were probably great expectations when ES cells were only thought of as immuno-privileged cells. But from more in in-depth investigations it appears that ES cell transplantation could at some point be identical to solid organ transplantation.

Several open questions were answered when Utermöhlen's group [5] described for the first time the role of a serine protease inhibitor expressed by ES cells, but also by some tumor cells. One of the answers being the fact that blastocysts are not rejected by the mother's immune system or why so few T cells infiltrate the site where ES cells are implanted. As already mentioned, serpin is important to the survival of ES cells. But what would happen if these ES cells were infected with pathogens? A serious problem would arise if NK cells or CTL started to kill infected adult stem cells. Guidotti et al. [51, 52] shed light on a very interesting property of CTLs, in that in a mouse model infected with hepatitis B virus (HBV) hepatocytes were not killed by CTLs; instead, without killing the infected cells, CTLs inhibited expression and replication of the HBV by secreting IFN-γ and TNF-α. But this process has still to be reproduced using infected ES cells.

Finally, the major point is to know the stage of development of the ES cells that are intended for transplantation. In the case of simple ES cells, the danger would lie in the derivation of unwanted cells. ES cells would probably

not launch an immune reaction, thanks to serpins, and the ES cells would survive. But in the case of progenitor cells, an immune reaction would be triggered due to the expression of ligands.

Obviously, it is necessary to conduct more investigations, but the concept that ES cells possess immune-privileged properties and are the ideal source of cells for transplantation has to be abandoned.

In fact, after so many years of evolution, there is no exception, the immune system is not blind and ES cells should be considered in the same light as tissue and organ from a genetically unrelated donor.

Acknowledgements JV and CdR are supported by the Swiss National Foundation for Research (CRSI33-125408) and the Swiss Parkinson Foundation. The authors are members of the Prometheus Consortium (Pluripotent stem cells for therapy of Parkinson's disease: a multidisciplinary and translational consortium).

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