The immune boundaries for stem cell based therapies: problems and prospective solutions

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Abstract

Stem cells have fascinated the scientific and clinical communities for over a century. Despite the controversy that surrounds this field, it is clear that stem cells have the potential to revolutionize medicine. However, a number of significant hurdles still stand in the way of the realization of this potential. Chiefly among these are safety concerns, differentiation efficiency and overcoming immune rejection. Here we review current progress made in this field to optimize the safe use of stem cells with particular emphasis on prospective interventions to deal with challenges generated by immune rejection.

Keywords: stem cells ● histocompatibility ● immune rejection ● cell therapies

Introduction

Recent years have seen enormous advances in the field of stem cell biology and applications thereof. The two unique properties of stem cells, those of developmental plasticity and extended proliferative potential in vitro, make them ideal candidates for use in cell replacement therapies. However, before such practical applications can be pursued, it is essential that we elucidate the fundamental mechanisms that govern their stable propagation and differentiation.

Despite the extensive use of bone marrow transplants for blood-related disorders, the concept of ‘stem cell therapies’ has only effectively been coined since the isolation of human embryonic stem cells (hESCs) [1]. Indeed, the link between stem cell research and the notion of ‘regenerative medicine’ did not appear in scientific literature until the late nineties. Ten years later, one of the most prolific, multi-disciplinary fields in recent scientific history has witnessed the first ‘adult’ stem cell (ASC)-based clinical
trials for a variety of conditions that have so far been considered incurable. These include Crohn’s disease [2], inflammatory bowel disease [3, 4], myocardial infarction [5, 6], graft-versus-host disease [7, 8] and a variety of autoimmune disorders [9–12]. Furthermore, the potential of ASCs to regenerate damaged tissue at so called ‘immunologically privileged’ sites is also being explored. Such studies include the restoration of neural function in neuroimmune/neurodegenerative conditions and in traumatic injury of the central nervous system [10, 13], as well as treatment of ocular lesions, autoimmune-related retinopathy and optic neuropathy [14].

The source of cells to be used for any given therapy is of major concern and must be addressed on a case-by-case basis. Ideally the cells should be easily accessible, pluripotent, genetically stable, exhibit high growth potential and not be subject to immunorejection. Differentiated cells can be autogenic but do not meet the pluripotency criterion and are not easily expanded. For these reasons the primary focus of cell transplantation therapies has been on embryonic stem cells (ESCs) and ASCs.

The regenerative potential of ESCs has now been successfully tested in several basic and preclinical studies. For instance, ESC-derived dopaminergic neurons were able to restore neuronal function in rats treated with 6-hydroxydopamine, a model for Parkinson’s disease [15–19]. Furthermore, ESCs differentiated along an oligodendrocyte lineage were able to induce central nervous system remyelination in rodents [16, 20]. Other examples include the regeneration of musculoskeletal tissue in mdx dystrophic mice [21, 22], infarcted myocardial tissue in mice [23, 24], rats [25] and pigs [26] and dysfunction rescue of pancreatic islet cells in streptozotocin-treated mice [27–31]. However, the widespread clinical use of ESCs cannot be achieved without first addressing inherent problems such as teratoma formation [32, 33] and graft rejection [11]. A further concern which applies to both ASCs and ESCs alike, is the issue of their accurate delivery to the site of injury. This is particularly problematic for the replacement of cells that lie deep in the brain, such as dopaminergic neurons [19].

Although the effectiveness of experimental therapies still needs to be ascertained, treatments using ASCs appear to have taken an early lead over those based on the use of ESCs. There are currently about 2250 clinical trials studies using ASCs versus only one using ESCs (see ‘www.clinicaltrial.gov’ for a worldwide registry).

In the short term, the use of immunosuppressive drugs is likely to be one of the main approaches used to prevent the immune rejection of stem cells (of either embryonic or adult origin). In this context, experience gained from half a century of research on solid organ transplantation will undoubtedly expedite the implementation of stem cell therapies [11, 34–36]. However, in the longer term, new assays and novel approaches must be developed to greatly aid efforts to understand and manipulate better, the molecular mechanisms governing immune tolerance [37–39]. In the meantime, a compromise approach would be to establish stem cell line banks that provide a wide range of human leucocyte antigen (HLA) profiles, thereby increasing the probability of a close match for a high percentage of potential recipients [40].

In addition to the direct therapeutic use of stem cells in tissue replacement, stem cells are advancing medicine in other ways. For instance, they are increasingly being used in drug screening processes [41, 42] providing cell-based systems to test the effects of potential new therapeutics. Pluripotent cells are particularly useful in this respect, as they can be induced to form all of the cells found in the adult, including those that are the targets of drugs under development. The advent of techniques for the generation of induced pluripotent stem (iPS) cells has also opened a new avenue of research whereby ESC-like cells can be derived from patients harbouring specific inheritable conditions, making it easier to study prospective treatments in vitro [43, 44].

Classification and sources of stem cells

All stem cells are defined by two unique properties, namely the capacity to self-renew or replicate in a non-differentiated state and the ability to differentiate into numerous cell types. One clear division between different types of stem cells is their origin: those derived from pre-implantation embryos are known as ESCs, whereas those identified in adult tissues are generally referred to as ASCs. Within these broad categories, stem cells can be further divided according to the number of differentiated cell types they give rise to.

The four predominant sources of stem cells are summarized in Table 1, namely, cells derived from pre-implantation embryos (material derived from leftover blastocysts from IVF), the foetus, the umbilical cord and adult tissues. Each of the different cell types will now be dealt with in turn.

Embyronic stem

ESC were initially isolated from early mouse blastocysts (at e3.5) [45, 46]. These blastocyst-derived stem cells epitomize the properties of stem cells and as such, their clinical potential for the regeneration of damaged/diseased tissue is now widely acknowledged.

Because ESCs were able to colonize the germ line of recipient embryos, they were initially used as tools for directed transgene and gene targeting [47]. The implementation of this powerful technology revolutionized the study of developmental biology, and spurred a decade-long quest for ESCs from species other than the mouse. Unfortunately, germ line transmission was never proven in any other species, and most efforts were abandoned after the first reports on nuclear transfer [48, 49], which offered a direct route for targeted transgenesis [50]. In contrast with the much-heralded pursuit of ESCs for biotechnological purposes, progress in defining the conditions for the isolation of primate ESCs [51] was seemingly more quiet, to the point that the breakthrough report on the derivation of the first hESCs took the scientific community almost by surprise [1].
Both in mouse and human, genes such as Oct3/4, Sox2 and Nanog are key factors that confer properties of pluripotency and self-renewal to these cells [52, 53] and subsequent differentiation requires the permanent down-regulation of these genes. In addition to the typical gene expression signature, hESCs also display a characteristic expression of cell surface markers and are negative for the stage specific embryonic antigen 1 (SSEA-1) and positive for SSEA-3, SSEA-4, TRA-1–60, TRA-1–81, telomerase and alkaline phosphatase [54]. The population doubling time of hESCs is very fast, ranging from 24 to 48 hrs [55]. Both in vitro (in the formation of embryoid bodies) and when injected into immunocompromised mice (in the formation of teratomas), hESCs give rise to derivatives of all three germ layers [1]. The addition of specific factors either singly or in combination results in the activation of particular differentiation pathways that can be assessed by the corresponding expression of markers for virtually every tissue of therapeutic interest. For these reasons, hESCs appear to be ideally positioned for widespread use in prospective regenerative therapies. However, their progression towards applications in the clinic has been somewhat slower than anticipated. This is largely because of concerns about safety, specifically the unknown potential of undifferentiated escapees to give rise to teratomas [32, 33, 56, 57]. A number of strategies have been proposed to address this problem, including improved screening [58] and the use of suicide genes [59–63]. A further concern is the risk of genomic instabilities which may accumulate as a result of long-term cell treatment.

### Table 1: Stem cell sources (human), types and potential immunoprivilege

| Source       | Tissue                                      | SC derived       | Immune privilege                                                                 | Refs.     | Pitfalls                                      |
|--------------|---------------------------------------------|------------------|----------------------------------------------------------------------------------|-----------|-----------------------------------------------|
| Embryonic    | Blastocyst (5–7 days)                       | ESCs             | Low expression MHC class I and MHC class II antigen                              | [36, 37, 156–160] | Not immune privileged                        |
|              | Gonadal ridge (6 weeks)                     |                  | Expression of mHC antigen and mitochondrial antigens (fragile immunoprivilege) |           | Patient-specific stem cell: (SCNT)           |
|              |                                              |                  | Inhibit allogenic T-cell proliferation                                           |           |                                               |
| Foetal       | Abortus (foetal tissues)                    | Foetal stem cells| Low expression MHC class I and MHC class II antigens                            | [161]     | Immuno-suppressive drugs                      |
| Newborn      | Umbilical cord blood                        | Umbilical Stem Cells | IL-10 synthesis                                                               | [162–164] |                                               |
|              | Wharton’s Jelly                             |                  | Expression of HLA-G                                                             |           |                                               |
|              | Placenta                                    |                  | Low expression of MHC class I and MHC class II antigens                          |           |                                               |
|              |                                              |                  | Secretion of immunosuppressive factors                                           |           |                                               |
| Adult        | Bone marrow                                | Hematopoietic stem cells | HLA matching                                                                  | [38, 95] | Risk of acute or chronic GVHD                |
|              | Peripheral blood                            |                  | Fastest engraftment                                                            |           |                                               |
|              | Bone marrow stroma                          | Mesenchymal stem cells | Production of immunosuppressive molecules                                      | [82–85]  |                                               |
|              | Fat, liposuction                            |                  | Inhibition of T cell                                                            |           |                                               |
|              | Others: epidermal (skin, hair) – Neuronal – Eye (limbo-corneal epithelium) – Muscle – Etc… | IPS              | Block proliferation and differentiation of B-lymphocytes                        | [165–167] | Patient-specific stem cell: (iPS)            |
|              |                                              |                  | Down-regulation of MHC class I and MCH class II antigens                        |           |                                               |
|              |                                              | NSC              | Up-regulation of αMSH and TGF-β2                                               |           |                                               |
|              |                                              | Muscle stem cells |                                                                                     |           |                                               |

**Note:** IPS: induced pluripotent stem cells; NSC: neural stem cells; MHC: major histocompatibility complex; mHC: minor histocompatibility complex; HLA: human leucocyte antigen; αMSH: α melanocyte-stimulating hormone; TGF-β2: transforming growth factor-β2; SCNT: somatic cell nuclear transfer; GVHD: graft versus host disease.
culture. Despite the first optimistic reports on the karyotypic stability of hESCs [1, 54, 64, 65], recent evidence strongly suggests that the adaptation of these cells to prolonged culture favours the development of chromosomal and sub-chromosomal alterations [66]. As the molecular mechanisms that control the in vitro proliferative adaptation and malignant transformation are fundamentally similar [67], the overall safety of hESC-based therapies must be thoroughly examined [68, 69]. For this reason, hESC-based clinical trials have been approached with more caution than those with ASCs. Despite these concerns, the US Food and Drug Administration (FDA) recently approved the first hESC-based clinical trials, designed to treat spinal cord injury [70]. Social pressure to develop cures for countless disorders has been fuelled by gross misrepresentations about the timeframe and scope of these treatments; however, the failed gene therapy trials in the 1990s must be a constant reminder of the dangers of rushing new therapies to the clinic.

**Adult stem cells**

ASCs encompass a variety of populations of undifferentiated cells and are found in most adult tissues, where they act as reservoirs during the normal turnover and regeneration of an organ or tissue. Both their potency and proliferative potential are typically narrower than those of their embryonic counterparts. Although the terminology is poorly defined and may encompass some overlap, ASCs should not be confused with tissue progenitor cells, which are found transiently at every stage of embryonic development. As every cell type in the body derives from the inner cell mass of the blastocyst (from which ESCs are obtained), it has been proposed that ASCs are remnants of original ESCs which remain in an undifferentiated state in specific niches [71, 72].

**Mesenchymal stem cells**

Mesenchymal stem cells are multipotent and are easily derived from a variety of tissues, including fat, skin and bone marrow. These cells are fibroblastic in appearance and can be expanded for many passages. Another typical feature of mesenchymal stem cells is their ability to differentiate along osteogenic, adipogenic and chondrogenic lineages [73]. It is still not clear whether these cells, which are mesodermal in origin, can give rise to true endodermal and ectodermal derivatives. One significant exception was the report of multipotent adult progenitor cells by Verfaillie [74], but difficulties in reproducing their methods has precluded the mainstream adoption of these cells as an alternative to hESCs. Because of the many reports on mesenchymal stem cells, which are derived from different origins, isolated and expanded in a variety of media and conditions, the International Society for Cellular Therapy established a set of minimal criteria for the definition of mesenchymal stem cells. These include the homogenous expression of the surface markers CD105, CD73 and CD90, as well as lack of haematopoietic markers such as CD45 and CD34 [73, 75]. Oct3/4 gene expression has occasionally been reported in mesenchymal stem cells [76, 77], although the role of this factor in maintaining self-renewal/pluripotency in ASCs has not yet been defined [78]. Mesenchymal stem cells are obvious candidates for regenerative therapies involving the reconstruction of connective tissues and wound healing [79, 80]. Particularly promising results have been reported for Crohn’s disease [2] and myocardial infarction [81]. However, the most intriguing characteristic of mesenchymal stem cells is their purported ability to act as immunomodulators [82–85] and pro-angiogenic agents [79, 86], the latter potentially mediated through the secretion of a variety of trophic signals that favour engraftment and/or endogenous regeneration [87]. A promising area of research involves the co-transplantation of mesenchymal stem cells and other tissues or cells, with an aim of providing a microenvironment that can support engraftment [88–92].

**Bone marrow and cord blood stem cells**

Haematopoietic stem cells represent less than 0.05% of the total bone marrow, but they have the potential to reconstitute all blood-forming lineages [93, 94]. Because of the enormous clinical implications of such ability, the haematopoietic stem cell compartment has historically been the best characterized stem cell niche. Haematopoietic stem cells can also be found in cord blood, which has been used for paediatric transplants even across major histocompatibility barriers [95]. More recently there has been interest in the haematopoietic niche as a source of stem cells for differentiation into non blood-related tissues [96, 97]. Again, because the haematopoietic compartment is derived from the mesoderm, there are substantive doubts that these cells can become bona fide endodermal or ectodermal derivatives. Because of the abundance of often contradictory reports, the jury is still out as to whether transplanted haematopoietic stem cells that migrate to target tissues, participate in their regeneration by actual differentiation, cell fusion or mere support of endogenous regeneration via revascularization [98].

**Amniotic fluid stem cells**

The latest addition to our repertoire of stem cells is amniotic fluid stem cells. The embryo is known to shed a variety of cells into the surrounding amniotic fluid during development [99, 100]. Very recently, multipotent cells with a multilineage differentiation potential that spans all three germ layers (endoderm, ectoderm and mesoderm) were clonally isolated and expanded for more than 250 population doublings [101]. These cells are unique in that they co-express markers typical of both mesenchymal stem cells (CD105, CD73 and CD90) and hESCs (SSEA-4, Oct3/4, telomerase and, in some lines, Tra-1–60). Because of the observation that they proliferate at a high rate without apparent loss of pluripotency or teratogenic potential when transplanted in immunodeficient animals, amniotic fluid stem cells were credited with being a safer alternative to hESCs. Such claims, however, may be premature and these results must be independently verified, with further work required to ascertain whether they really do have the same degree of pluripotency as hESCs.
Limitations of stem cells

Based on some of the issues already discussed, it has been proposed that stem cell research be limited to ASCs. However, the counter argument is that ESCs have the highest potential to become a clinical reality [102]. The most important differences between embryonic and ASCs are highlighted in Table 2. Fundamental research is required to define and understand the immunological features of stem cells and improve their delivery and homing processes.

ESCs can be maintained in culture in an undifferentiated state for prolonged periods without losing their ability to differentiate. However, the very same properties that make them so appealing could also present us with formidable obstacles [103, 104]. For instance, their unlimited differentiation capability might stand in the way of their efficient differentiation into specific cell types. Another way of looking at this problem is that there are many more steps between a completely undifferentiated cell and a mature tissue than we would otherwise see between an 'adult' progenitor and its differentiated progeny. As we are limited in our capacity to mimic the exquisitely complex nature of the cellular microenvironments where native differentiation takes place, a common observation in ESC-based differentiation protocols is a cumulative loss of efficiency in every step. As for their other property (unlimited proliferation), we have already discussed how even minute traces of undifferentiated cells in transplantable preparations might lead to the development of teratomas in vivo.

ASCs typically do not exhibit these limitations, but are less versatile, proliferate at a much slower pace, senesce quickly and have proven to be very difficult to maintain in an undifferentiated state in culture. These cells have been shown to differentiate into a variety of tissues of great therapeutic value, and their potential isolation and expansion from the same patient who is to be treated offers a direct way for autologous therapies without the need for reprogramming or application of immunosuppressive drugs.

### Table 2 Comparison between embryonic and ASCs

| Stem cells type | Advantages | Disadvantages/pitfalls |
|-----------------|------------|------------------------|
| **Embryonic**   | Pluripotent | Difficult to keep stable and undifferentiated in culture |
|                 | High number of cells: can be expanded indefinitely and in an undifferentiated state [51, 54, 55, 168] | Complicated to maintain in a feeder-free state |
|                 | Self-renewing capacity [54, 169] | Risk of xenogenic contamination [171, 172] |
|                 | Patient specific cell-based therapies to reach a low immunogenicity: SCNT strategies [108, 170] | Requirement for novel protocol with defined medium, and replacement of animal proteins by human ones [173] |
|                 | Risk of tumour formation for transplant therapy [1, 33] | Requirement for pure differentiated cell population |
|                 | Epigenetic instability [174] and chromosomal abnormalities [66] | Slow progression towards clinical applications [70] |
|                 | Ethical concerns [124, 170, 175] | |
| **Adult**       | Diverse sources available | Culture limitations: slow growth, do not self-renew, low number, difficult to produce, differentiate easily in vitro |
|                 | Adult tissues; does not involve the destruction of human embryos | Need to immortalize to generate high number of cells (e.g. transfection of neural progenitor cells with retrovirus encoding hTER [177]) |
|                 | Easily isolated | Specimens, from which some stem cell types are derived, require small surgery to isolate (bone marrow aspirates, lipoaspirate, biopsy specimens) |
|                 | Autologous, low immunogenicity (suitable for allografts) [2, 3, 8, 82, 83, 85, 90, 142, 143, 176] | High cost to expand for clinical use (need of good manufacturing practice installation) [105, 106] |
|                 | Some cell types are prolific, lack of genetic marker that causes immune rejection and escape to ethical and legal concerns (e.g. umbilical cord stem cells [124]) | Plasticity criteria are controversial and inconsistent: rare transformation events and cell fusion with host cells need to be excluded [178, 179] |
|                 | Patient specific cell-based therapies: iPS [43, 44, 170] | Trans-differentiation, de-differentiation and unexpected plasticity may be because of aberrant processes [180] |
|                 | Largely used in clinical trials [2] (www.clinicaltrial.gov) | Lack of consistent pluripotency assay [181, 182] |
However, ASCs are scarce and/or difficult to find and expand. Because their isolation and expansion requires the use of good manufacturing practice techniques, the costs involved might end up being prohibitive for many institutions [105, 106].

Alternatives to overcome the limitations of stem cells

It is now generally believed that hESCs might represent an unlimited source from which to derive tissues for regenerative medicine [1, 2]. Coupled to somatic cell nuclear transfer (SCNT) [48], hESC technologies also opened the door to the possibility of generating tissues genetically identical to those of the donor. The principle of this application, also termed ‘therapeutic cloning’, has already been proven in mice [49] and non-human primates [51]. The generation of hESCs from SCNT-derived embryos, however, has remained elusive. Reports to that effect by Hwang et al. [107–109] turned out to be fraudulent, and had to be retracted [110]. To date, no hESCs have been derived by SCNT, despite progress at obtaining human blastocysts [111].

Technical, logistic and ethical concerns (not to be discussed here) led to a quest for methods to reprogram somatic cells without the use of nuclear transfer. Of particular note were strategies that made use of cellular extracts [112–115] or fusion with ESCs [116]. However, none of these provided a definitive solution to the problem. A different approach was used to achieve success. By delivering constitutively active copies of critical regulators of hESC homeostasis (Oct3/4, Sox2, c-Myc and Klf4), Takahashi and coworkers were able to reprogram adult fibroblasts into hES-like cells (induced pluripotent cells or iPScs), first in mice [117] and in shortly thereafter in human beings [118]. Similar results were independently reported by another team using a slightly different set of transcription factors (Oct3/4, Sox2, Nanog and LIN28) [119].

Not surprisingly, these studies were hailed as a major biological breakthrough. A method that was both conceptually and technically simple had been sufficient to reprogram differentiated cells from adult donors without the use of human embryos. The potential therapeutic implications were subsequently realized by Hanna and coworkers, who treated sickle cell anaemia in mice by autologous transplantation of iPSc-derived haematopoietic progenitors [120].

A caveat of these procedures, however, was that the gene combinations required for reprogramming were delivered using retroviral vectors; thus rendering the resulting iPSc cells unusable for clinical purposes because of the risks of virus insertional mutagenesis and the potential subsequent reactivation of the reprogramming genes [121]. The recent report on the generation of iPSc cells in a virus-free system addresses a critical safety concern for their potential use in regenerative medicine [122, 123]. Moreover, and as mentioned before, iPSc cells could represent an unmatched option for the production of patient-specific stem cells for therapy without the problem of immune rejection. However, iPSc cells have been shown to be functionally equivalent to hESCs, and, as such, still have the same limitations regarding teratogenic potential and inefficient differentiation. These concerns have prompted many researchers to focus on ASCs, because they have a wide range of sources and are generally considered safer. In this context, umbilical cord blood stem cells have been favoured not only for their therapeutic and scientific value, but also because they escape many of the safety, legal and ethical considerations that have hampered hESC research so far [124].

Histocompatibility and immune rejection

In vivo engraftment and function of human stem cells is compromised by potential immunological barriers [125]. One of these barriers is the expression of HLAs on stem cells [126]. Major histocompatibility complex (MHC) class I and II HLA antigens are master triggers of robust immunological rejection of grafts because they present antigens to T lymphocytes. Class I HLA molecules are crucial in presenting tumor antigens and viral antigens on the surface of infected cells to cytotoxic T lymphocytes (CTL). Specific CTLs can efficiently lyse a target cell upon recognition of MHC class I-presented antigen on the cell surface. Although undifferentiated hESCs do not express class II HLA molecules, they do exhibit some expression of the class I type. However, expression of class I HLA antigens increases with differentiation [127, 128]. Such an increase in expression is even more pronounced under infectious and inflammatory conditions where interferon (IFN)-γ is abundant [129]. hESCs and their differentiated progeny are, therefore, similar to any other nucleated cell of the human body with regard to their class I HLA expression, and can be recognized as foreign and targeted by CTL. In addition to MHC molecules, hESCs also express minor histocompatibility complex (mHC) antigens [125]. Although these are not as immunogenic as MHC antigens, they are sufficient to drive the immune system to reject the graft [130].

Graft rejection can also be mediated through a mismatch in the ABO blood group system (ABO) antigens between the donor and the recipient [131] because of their high immunogenicity. To date, the ABO antigen expression status of hESCs and their differentiated derivatives is still unknown. Nevertheless, and as mentioned above, the generation of ‘universal donor’ pluripotent cells [132], patient-specific stem cells and ABO compatible hESCs would also solve the problem [133].

Are ESCs immunogenic or immunopriviliged?

Immunogenicity and immune privilege at a given graft site are mutually exclusive, and their onset is dictated by a variety of factors on both donor’s and recipient’s sides. On one hand (as
described earlier), HLA class I antigen expression is very low on hESCs but can rise to those levels found on adult cells if differentiated or exposed to IFN-γ [126–129], resulting in strong immune responses that can lead to graft rejection. Furthermore, gene expression profiling [129] has shown that hESCs do not express transforming growth factor (TGF-β), CD95L and interleukin (IL)-10, all of which are operative in immune privileged sites to protect the graft from immune attacks. In contrast to the expression of class I antigens, however, hESCs do not express HLA class II, nor the co-stimulatory molecules, such as CD80 and CD86 [128]. The presence of co-stimulatory receptors on antigen presenting cells is crucial for the HLA class II molecules to stimulate CD4 T lymphocytes following antigen presentation. Absence of co-stimulatory molecules leads to the anergy of T cells [134] and their inability to fully exert their effector function. Recognition of hESCs by natural killer (NK) lysis receptors is low or absent, and only a low level of killing can be achieved when hESCs are incubated with NK cells [126]. Few studies have been done on the immunogenicity of stem cells; this is especially true of NKT cells, whose role is now emerging in the field of organ transplantation. NKT cells are cells with potent immunomodulatory properties, that have been implicated in allograft tolerance [135, 136] and therefore can be used in monotherapy or combination immunotherapy of cell transplant rejection [122, 137, 138]. Therefore, strategies to assess the ability of ESCs to induce immune tolerance through NKT cells obtained from ESCs are highly sought after [138]. It is clear that not all conditions that would provide immune privilege to hESCs are met (Table 1), and that using hESCs in transplantation medicine needs additional interventions to establish immune tolerance to the graft.

Overcoming the need for immune manipulation to avoid rejection

There are a few instances where stem cells could be used successfully to regenerate tissues without the need to silence the immune system. In 2005, Miki and coworkers succeeded in isolating human amnion epithelial cells with ESC-like properties [139, 140]. This research suggests that the amnion, derived from term placentas after live birth, may be a useful and non-controversial source of stem cells for use in transplantation. Because these cells are a genetic match to the developing foetus, tissues derived from them in vitro will escape immune rejection if used to treat defects in that newborn. Cryopreservation of cells is also an option to provide a personalized tissue bank for use by the donor later in life.

With our current knowledge (as discussed earlier), it is possible to obtain ASCs from various tissues (Table 1) and expand them in vitro. This ability could signal the onset of a new era for regenerative medicine, whereby stem cells could be obtained from a patient, expanded in vitro and transplanted back in to the same patient without running the risk of immune rejection. However, in some instances the affected tissue may not harbour sufficient numbers of unaffected stem cells to allow isolation.

One example is diabetes mellitus type 1, where the autoimmune destruction of insulin-producing β cells of the pancreas is often total and permanent [11].

An attractive source for immunoprivileged ASCs is the mesenchymal stem cell. Horwitz and coworkers [141] demonstrated that allogeneic mesenchymal stem cells could be safely administered to children with severe osteogenesis imperfecta, where they engraft in genetically defective bone, and differentiate to osteoblasts capable of extending the clinical benefits of bone marrow transplantation. One plausible explanation for the successful engraftment of mesenchymal stem cells is their ability to suppress T lymphocytes through the release of soluble mediators such as TGF-β, indoleamine 2,3-dioxygenase and prostaglandin E2 [142]. Their immunosuppressive properties favour engraftment despite their expression of HLA class I and the possibility of expression of HLA class II upon IFN-γ treatment [143].

Manipulating immune tolerance for stem cell-based therapies

The mechanisms leading to the removal of immune responsiveness, or tolerance, to a given antigen takes place in the thymus (central tolerance) or in the periphery (peripheral tolerance). T cells specific for ‘self’-antigens are either deleted in the thymus or driven into a state of anergy in the periphery as a result of insufficient costimulation and regulatory T-cell induction.

In the periphery, T cells can be driven into tolerance by the presence of tolerogenic dendritic cells (DC) or Treg. Tolerogenic DCs do not possess the full machinery required to stimulate T cells, rather, they induce anergy. Tolerogenic DCs have been shown to prolong allograft survival [144]. Given their remarkable functional plasticity, DCs are easily rendered tolerogenic by culturing them with mesenchymal stem cells [145]. Treg, by virtue of their strong immunosuppressive potential on T cells, are crucial for establishing immune tolerance to self-antigens and preventing autoimmunity [146]. Joffre and coworkers used mouse models of bone marrow, skin and cardiac allografts in which they achieved lifelong immunological tolerance following treatment with alloantigen-stimulated regulatory T cells [34]. A reduction in the size of the Treg population can result in graft rejection. An example of a mouse model of allogeneic skin transplantation was recently reported, following treatment with an agonist monoclonal antibody that targets the Tim-1 receptor [147]. Activation of naive T cells through the Tim-1 receptor appears to favour Th1 and Th17 differentiation over Treg, which explains the rejection of the graft.

To date, successful tolerance of a graft in human beings still requires continuous suppression of alloantigen-specific T cells in the periphery. However, immunosuppression regimens would not be necessary if it were possible to delete antigen-specific T cells before they exit the thymus. Although tempting, the idea of inducing immune tolerance through manipulating the thymus faces numerous challenges, including thymus atrophy that starts at
puberty [148] and foreign gene delivery to the thymus. The latter obstacle can be overcome by transplantation of haematopoietic stem cells, which have been shown to lead to donor antigen presenting cell driven depletion of donor-reactive T cells in the thymus and subsequent acceptance of allogeneic grafts in the mouse [149, 150]. Study in human was achieved by Kawai and coworkers, who combined HLA-mismatched bone marrow and kidney transplantation, and reported a stable renal-allograft function after complete withdrawal of immunosuppressive drugs. However, this author appoints that the results of attempts to extend the immune tolerance from animal models (mice and monkey) to human beings have been disappointing [151]. Enhanced production of lymphoid progenitor cells following transplantation was also achieved by administration of thymic growth factors such as IL-7 [152] and FLT3 ligand [153]. Rejuvenation of the thymus through blockade of sex steroids has been demonstrated in patients with prostate cancer [154] and in castrated mice [155]. Interestingly, thymus rejuvenation proved beneficial for the engraftment of haematopoietic stem cells, which paralleled an increase in thymic T cells [38].

Conclusions

Increasing experimental evidence has given credibility to the claim that stem cell research could change the future of medicine. However, a view of stem cell therapies as merely a replacement tool would be rather narrow. In the case of autoimmune diseases, for instance, replenishing the damaged cells would be transient at most, as the immune machinery that destroyed the tissue in the first place would remain intact. It is now accepted that the re-education of the immune system, be it to correct an autoimmune disorder or to prevent allorejection, is within our reach. In this context, stem cell therapies must be designed in a comprehensive manner to account for both the replacement of the damaged tissue and the prevention of additional destruction without systemic immunosuppression.

Several approaches to re-establish immune tolerance are either ready for clinical application or will be translated in the near future from promising experimental in vivo results. Long-term allograft survival might be improved by concomitant implementation of tolerance-inducing protocols. This would represent a major step to avoid the current approach of using general immunosuppression, which is rather thought to interfere with the establishment of tolerance. The dimension of the problem requires a combination of expertise that will not be realistically found in any given group. A worldwide, multidisciplinary effort involving experts in regenerative medicine, stem cell biology and induction of immunological tolerance, will be necessary to translate basic findings in a rapid but safe manner.

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