 J. Cell. Mol. Med. Vol 13, No 8A, 2009 pp. 1464–1475

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

Hmadcha Abdelkrim [a, *], Domínguez-Bendala Juan [b], Wakeman Jane [c], Arredouani Mohamed [d], Soria Bernat [a]

[a] *Department of Cell Therapy and Regenerative Medicine, Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Sevilla, Spain*
[b] *Pancreatic Development & Stem Cell Laboratory, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA*
[c] *North West Cancer Research Fund Institute, School of Biological Sciences, University of Wales Bangor, Memorial Building, Gwynedd, UK*
[d] *Department of Surgery, Beth Israel Deaconess Medical Center, Boston, MA, USA*

Received: February 27, 2009; Accepted: June 25, 2009

- **Introduction**
- **Classification and sources of stem cells**
  - **Embryonic stem**
  - **Adult stem cells**
    - **Mesenchymal stem cells**
    - **Bone marrow and cord blood stem cells**
    - **Amniotic fluid stem cells**
- **Limitations of stem cells**
- **Alternatives to overcome the limitations of stem cells**
- **Histocompatibility and immune rejection**
- **Are ESCs immunogenic or immunoprivileged?**
- **Overcoming the need for immune manipulation to avoid rejection**
- **Manipulating immune tolerance for stem cell-based therapies**
- **Conclusions**

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

Translational therapies and new approaches for the treatment of immune-based disorders Review Series.

*Correspondence to: Abdelkrim HMADCHA,
Department of Cell Therapy and Regenerative Medicine,
Andalusian Center for Molecular Biology and Regenerative Medicine,
(CABIMER), Avenida Americo Vespucio s/n,
Parque Cientifico y Tecnológico Cartuja 93, 41092–Sevilla, Spain.
Tel.: +34-954 468 373
Fax: +34-954 461 664
E-mail: karim.hmadcha@cabimer.es

doi:10.1111/j.1582-4934.2009.00837.x

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

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 trial 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.

Embryonic stem

ESCs 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 transgenesis 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].

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

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 immunoprovilege)		Patient-specific stem cell: (SCNT)
			Inhibit allogenic T-cell proliferation		ABO compatible hESCs
					Immuno-suppressive drugs
Foetal	Abortus (foetal tissues)	Foetal stem cells	Low expression MHC class I and MHC class II antigens	[161]	
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		
			Autogous/allogeneic transplantation		
	Bone marrow stroma	Mesenchymal stem cells	Production of immunosuppressive molecules	[82–85]	
	Fat, liposuction		Inhibition of T cell		
			Block proliferation and differentiation of B-lymphocytes		
	Others: epidermal (skin, hair) – Neuronal – Eye (*limbo-corneal epithelium*) – Muscle – Etc…	iPS	Down-regulation of MHC class I and MCH class II antigens	[165–167]	Patient-specific stem cell: (iPS)
		NSC	Up-regulation of αMSH and TGF-β_2		
		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-β_2: transforming growth factor-β_2; SCNT: somatic cell nuclear transfer; GVHD: graft *versus* host disease.

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 gem 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

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

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 blastocysts (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 homogeneous 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.

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

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 stat [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]

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.

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

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 iPS), 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 iPS-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 iPS 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 iPS 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, iPS cells could represent an unmatched option for the production of patient-specific stem cells for therapy without the problem of immune rejection. However, iPS 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 immunoprivileged?

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

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

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 coworker*s* [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 E_2 [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

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

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 immunosuppresion.

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.

Acknowledgements

This work was supported by the Consejería de Salud (Junta de Andalucía) and Fundación Progreso y Salud, the Spanish Ministry of Health, Red TerCel, North West Cancer Research Fund and Diabetes Research Institute Foundation.

References

1. **Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al.*** Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282: 1145–7.
2. **Garcia-Olmo D, Garcia-Arranz M, Herreros D, *et al.*** A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum*. 2005; 48: 1416–23.
3. **Fortun PJ, Hawkey CJ.** The role of stem cell transplantation in inflammatory bowel disease. *Autoimmunity*. 2008; 41: 654–9.
4. **Lanzoni G, Roda G, Belluzzi A, *et al.*** Inflammatory bowel disease: moving toward a stem cell-based therapy. *World J Gastroenterol*. 2008; 14: 4616–26.
5. **Assmus B, Honold J, Schachinger V, *et al.*** Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med*. 2006; 355: 1222–32.
6. **Schachinger V, Erbs S, Elsasser A, *et al.*** Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med*. 2006; 355: 1210–21.
7. **Fang B, Song YP, Liao LM, *et al.*** Treatment of severe therapy-resistant acute graft-versus-host disease with human adipose tissue-derived mesenchymal stem cells. *Bone Marrow Transplant*. 2006; 38: 389–90.
8. **Le Blanc K, Rasmusson I, Sundberg B, *et al.*** Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet*. 2004; 363: 1439–41.
9. **Burt RK, Marmont A, Oyama Y, *et al.*** Randomized controlled trials of autologous hematopoietic stem cell transplantation for autoimmune diseases: the evolution from myeloablative to lymphoablative transplant regimens. *Arthritis Rheum*. 2006; 54: 3750–60.
10. **Voltarelli JC, Couri CE, Stracieri AB, *et al.*** Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*. 2007; 297: 1568–76.
11. **Kabelitz D, Geissler EK, Soria B, *et al.*** Toward cell-based therapy of type I diabetes. *Trends Immunol*. 2008; 29: 68–74.
12. **Tateishi-Yuyama E, Matsubara H, Murohara T, *et al.*** Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet*. 2002; 360: 427–35.
13. **Chen Z, Palmer TD.** Cellular repair of CNS disorders: an immunological perspective. *Hum Mol Genet*. 2008; 17: R84–92.
14. **Otani A, Dorrell MI, Kinder K, *et al.*** Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. *J Clin Invest*. 2004; 114: 765–74.
15. **Barberi T, Klivenyi P, Calingasan NY *et al.*** Neural subtype specification of fertilization and nuclear transfer embryonic

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

stem cells and application in parkinsonian mice. *Nat Biotechnol*. 2003; 21: 1200–7.

16. **Bjorklund LM, Sanchez-Pernaute R, Chung S, *et al.*** Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci USA*. 2002; 99: 2344–9.
17. **Sonntag KC, Sanchez-Pernaute R.** Tailoring human embryonic stem cells for neurodegenerative disease therapy. *Curr Opin Investig Drugs*. 2006; 7: 614–8.
18. **Spitere K, Toulouse A, O'Sullivan DB *et al.*** TAT-PAX6 protein transduction in neural progenitor cells: a novel approach for generation of dopaminergic neurones *in vitro*. *Brain Res*. 2008; 1208: 25–34.
19. **Svendsen C.** Stem cells and Parkinson's disease: toward a treatment, not a cure. *Cell Stem Cell*. 2008; 2: 412–3.
20. **Brustle O, Jones KN, Learish RD, *et al.*** Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science*. 1999; 285: 754–6.
21. **Darabi R, Gehlbach K, Bachoo RM, *et al.*** Functional skeletal muscle regeneration from differentiating embryonic stem cells. *Nat Med*. 2008; 14: 134–43.
22. **Darabi R, Santos FN, Perlingeiro RC.** The therapeutic potential of embryonic and adult stem cells for skeletal muscle regeneration. *Stem Cell Rev*. 2008; 4: 217–25.
23. **Klug MG, Soonpaa MH, Field LJ.** DNA synthesis and multinucleation in embryonic stem cell-derived cardiomyocytes. *Am J Physiol*. 1995; 269: H1913–21.
24. **Klug MG, Soonpaa MH, Koh GY, *et al.*** Genetically selected cardiomyocytes from differentiating embronic stem cells form stable intracardiac grafts. *J Clin Invest*. 1996; 98: 216–24.
25. **Laflamme MA, Chen KY, Naumova AV, *et al.*** Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol*. 2007; 25: 1015–24.
26. **Kehat I, Khimovich L, Caspi O, *et al.*** Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol*. 2004; 22: 1282–9.
27. **Kroon E, Martinson LA, Kadoya K, *et al.*** Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nat Biotechnol*. 2008; 26: 443–52.
28. **D'Amour KA, Bang AG, Eliazer S, *et al.*** Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol*. 2006; 24: 1392–401.
29. **Leon-Quinto T, Jones J, Skoudy A, *et al.*** *In vitro* directed differentiation of mouse embryonic stem cells into insulin-producing cells. *Diabetologia*. 2004; 47: 1442–51.
30. **Soria B, Roche E, Berna G, *et al.*** Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes*. 2000; 49: 157–62.
31. **Vaca P, Martin F, Vegara-Meseguer JM, *et al.*** Induction of differentiation of embryonic stem cells into insulin-secreting cells by fetal soluble factors. *Stem Cells*. 2006; 24: 258–65.
32. **Bieberich E, Silva J, Wang G, *et al.*** Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. *J Cell Biol*. 2004; 167: 723–34.
33. **Fujikawa T, Oh SH, Pi L, *et al.*** Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. *Am J Pathol*. 2005; 166: 1781–91.
34. **Joffre O, Santolaria T, Calise D, *et al.*** Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. *Nat Med*. 2008; 14: 88–92.
35. **Scandling JD, Busque S, Dejbakhsh-Jones S, *et al.*** Tolerance and chimerism after renal and hematopoietic-cell transplantation. *N Engl J Med*. 2008; 358: 362–8.
36. **Swijnenburg RJ, Schrepfer S, Govaert JA, *et al.*** Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proc Natl Acad Sci USA*. 2008; 105: 12991–6.
37. **Chidgey AP, Boyd RL.** Immune privilege for stem cells: not as simple as it looked. *Cell Stem Cell*. 2008; 3: 357–8.
38. **Sutherland JS, Spyroglou L, Muirhead JL, *et al.*** Enhanced immune system regeneration in humans following allogeneic or autologous hemopoietic stem cell transplantation by temporary sex steroid blockade. *Clin Cancer Res*. 2008; 14: 1138–49.
39. **Waldmann H, Cobbold S.** Exploiting tolerance processes in transplantation. *Science*. 2004; 305: 209–12.
40. **Carpenter MK, Rosler ES, Fisk GJ, *et al.*** Properties of four human embryonic stem cell lines maintained in a feeder-free culture system. *Dev Dyn*. 2004; 229: 243–58.
41. **Festag M, Sehner C, Steinberg P, *et al.*** An *in vitro* embryotoxicity assay based on the disturbance of the differentiation of murine embryonic stem cells into endothelial cells. I: establishment of the differentiation protocol. *Toxicol In Vitro*. 2007; 21: 1619–30.
42. **Fico A, Manganelli G, Simeone M, *et al.*** High-throughput screening-compatible single-step protocol to differentiate embryonic stem cells in neurons. *Stem Cells Dev*. 2008; 17: 573–84.
43. **Dimos JT, Rodolfa KT, Niakan KK, *et al.*** Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008; 321: 1218–21.
44. **Park IH, Arora N, Huo H, *et al.*** Disease-specific induced pluripotent stem cells. *Cell*. 2008; 134: 877–86.
45. **Evans MJ, Kaufman MH.** Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981; 292: 154–6.
46. **Martin GR.** Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA*. 1981; 78: 7634–8.
47. **Capecchi MR.** Altering the genome by homologous recombination. *Science*. 1989; 244: 1288–92.
48. **Campbell KH, McWhir J, Ritchie WA, *et al.*** Sheep cloned by nuclear transfer from a cultured cell line. *Nature*. 1996; 380: 64–6.
49. **Wilmut I, Schnieke AE, McWhir J, *et al.*** Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997; 385: 810–3.
50. **McCreath KJ, Howcroft J, Campbell KH, *et al.*** Production of gene-targeted sheep by nuclear transfer from cultured somatic cells. *Nature*. 2000; 405: 1066–9.
51. **Thomson JA, Marshall VS.** Primate embryonic stem cells. *Curr Top Dev Biol*. 1998; 38: 133–65.
52. **Bhattacharya B, Miura T, Brandenberger R, *et al.*** Gene expression in human embryonic stem cell lines: unique molecular signature. *Blood*. 2004; 103: 2956–64.
53. **Boyer LA, Lee TI, Cole MF, *et al.*** Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*. 2005; 122: 947–56.
54. **Amit M, Carpenter MK, Inokuma MS *et al.*** Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev Biol*. 2000; 227: 271–8.
55. **Cowan CA, Klimanskaya I, McMahon J *et al.*** Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med*. 2004; 350: 1353–6.
56. **Hori Y, Rulifson IC, Tsai BC, *et al.*** Growth inhibitors promote differentiation of insulin-producing tissue from embryonic

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

stem cells. *Proc Natl Acad Sci USA*. 2002; 99: 16105–10.
57. **Odorico JS, Kaufman DS, Thomson JA.** Multilineage differentiation from human embryonic stem cell lines. *Stem Cells*. 2001; 19: 193–204.
58. **Vogel G.** Cell biology. Ready or not? Human ES cells head toward the clinic. *Science*. 2005; 308: 1534–8.
59. **Fareed MU, Moolten FL.** Suicide gene transduction sensitizes murine embryonic and human mesenchymal stem cells to ablation on demand – a fail-safe protection against cellular misbehavior. *Gene Ther*. 2002; 9: 955–62.
60. **Schuldiner M, Itskovitz-Eldor J, Benvenisty N.** Selective ablation of human embryonic stem cells expressing a "suicide" gene. *Stem Cells*. 2003; 21: 257–65.
61. **Tamada K, Wang XP, Brunicardi FC.** Molecular targeting of pancreatic disorders. *World J Surg*. 2005; 29: 325–33.
62. **Wang XP, Yazawa K, Yang J, *et al.*** Specific gene expression and therapy for pancreatic cancer using the cytosine deaminase gene directed by the rat insulin promoter. *J Gastrointest Surg*. 2004; 8: 98–108.
63. **Yazawa K, Fisher WE, Brunicardi FC.** Current progress in suicide gene therapy for cancer. *World J Surg*. 2002; 26: 783–9.
64. **Carpenter MK, Rosler E, Rao MS.** Characterization and differentiation of human embryonic stem cells. *Cloning Stem Cells*. 2003; 5: 79–88.
65. **Rosler ES, Fisk GJ, Ares X, *et al.*** Long-term culture of human embryonic stem cells in feeder-free conditions. *Dev Dyn*. 2004; 229: 259–74.
66. **Imreh MP, Gertow K, Cedervall J, *et al.*** *In vitro* culture conditions favoring selection of chromosomal abnormalities in human ES cells. *J Cell Biochem*. 2006; 99: 508–16.
67. **Baker DE, Harrison NJ, Maltby E, *et al.*** Adaptation to culture of human embryonic stem cells and oncogenesis *in vivo*. *Nat Biotechnol*. 2007; 25: 207–15.
68. **Borstlap J, Kurtz A, Stacey G, *et al.*** Development of a European human embryonic stem cell registry. *Regen Med*. 2008; 3: 945–51.
69. **Hyun I, Lindvall O, Ahrlund-Richter L *et al.*** New ISSCR guidelines underscore major principles for responsible translational stem cell research. *Cell Stem Cell*. 2008; 3: 607–9.
70. **Wadman M.** Stem cells ready for prime time. *Nature*. 2009; 457: 516.
71. **Anderson DJ, Gage FH, Weissman IL.** Can stem cells cross lineage boundaries? *Nat Med*. 2001; 7: 393–5.
72. **van der Kooy D, Weiss S.** Why stem cells? *Science*. 2000; 287: 1439–41.
73. **Dominici M, Le Blanc K, Mueller I, *et al.*** Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; 8: 315–7.
74. **Verfaillie CM.** Multipotent adult progenitor cells: an update. *Novartis Found Symp*. 2005; 265: 55–61.
75. **Horwitz EM, Le Blanc K, Dominici M *et al.*** Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy*. 2005; 7: 393–5.
76. **D'Ippolito G, Diabira S, Howard GA, *et al.*** Low oxygen tension inhibits osteogenic differentiation and enhances stemness of human MIAMI cells. *Bone*. 2006; 39: 513–22.
77. **D'Ippolito G, Howard GA, Roos BA, *et al.*** Isolation and characterization of marrow-isolated adult multilineage inducible (MIAMI) cells. *Exp Hematol*. 2006; 34: 1608–10.
78. **Liedtke S, Stephan M, Kogler G.** Oct4 expression revisited: potential pitfalls for data misinterpretation in stem cell research. *Biol Chem*. 2008; 389: 845–50.
79. **Wu Y, Chen L, Scott PG, *et al.*** Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells*. 2007; 25: 2648–59.
80. **Wu Y, Wang J, Scott PG, *et al.*** Bone marrow-derived stem cells in wound healing: a review. *Wound Repair Regen*. 2007; 15: S18–26.
81. **Hare JM, Chaparro SV.** Cardiac regeneration and stem cell therapy. *Curr Opin Organ Transplant*. 2008; 13: 536–42.
82. **Abdi R, Fiorina P, Adra CN, *et al.*** Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes*. 2008; 57: 1759–67.
83. **Le Blanc K, Ringden O.** Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med*. 2007; 262: 509–25.
84. **Mishra PK.** Bone marrow-derived mesenchymal stem cells for treatment of heart failure: is it all paracrine actions and immunomodulation? *J Cardiovasc Med*. 2008; 9: 122–8.
85. **Ozaki K, Sato K, Oh I, *et al.*** Mechanisms of immunomodulation by mesenchymal stem cells. *Int J Hematol*. 2007; 86: 5–7.
86. **Ball SG, Shuttleworth CA, Kielty CM.** Mesenchymal stem cells and neovascularization: role of platelet-derived growth factor receptors. *J Cell Mol Med*. 2007; 11: 1012–30.
87. **Sadat S, Gehmert S, Song YH, *et al.*** The cardioprotective effect of mesenchymal stem cells is mediated by IGF-I and VEGF. *Biochem Biophys Res Commun*. 2007; 363: 674–9.
88. **Ball LM, Bernardo ME, Roelofs H, *et al.*** Cotransplantation of *ex vivo* expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. *Blood*. 2007; 110: 2764–7.
89. **Johansson U, Rasmusson I, Niclou SP *et al.*** Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization. *Diabetes*. 2008; 57: 2393–401.
90. **Fang B, Li N, Song Y, *et al.*** Cotransplantation of haploidentical mesenchymal stem cells to enhance engraftment of hematopoietic stem cells and to reduce the risk of graft failure in two children with severe aplastic anemia. *Pediatr Transplant*. 2009; 13: 499–502.
91. **Lee ST, Maeng H, Chwae YJ, *et al.*** Effect of mesenchymal stem cell transplantation on the engraftment of human hematopoietic stem cells and leukemic cells in mice model. *Int J Hematol*. 2008; 87: 327–37.
92. **Noort WA, Kruisselbrink AB, in't Anker PS, *et al.*** Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. *Exp Hematol*. 2002; 30: 870–8.
93. **Becker AJ, McCulloch EA, Till JE.** Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*. 1963; 197: 452–4.
94. **Morrison SJ, Uchida N, Weissman IL.** The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol*. 1995; 11: 35–71.
95. **Hong DS, Deeg HJ.** Hemopoietic stem cells: sources and applications. *Med Oncol*. 1994; 11: 63–8.
96. **Harris DT, Badowski M, Ahmad N, *et al.*** The potential of cord blood stem cells for use in regenerative medicine. *Expert Opin Biol Ther*. 2007; 7: 1311–22.
97. **Harris DT, Rogers I.** Umbilical cord blood: a unique source of pluripotent stem cells for regenerative medicine. *Curr Stem Cell Res Ther*. 2007; 2: 301–9.
98. **Iskovich S, Kaminitz A, Yafe MP, *et al.*** Participation of adult bone marrow-derived stem cells in pancreatic regeneration: neogenesis *versus* endogenesis. *Curr Stem Cell Res Ther*. 2007; 2: 272–9.

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

99. **Polgar K, Adany R, Abel G, *et al.*** Characterization of rapidly adhering amniotic fluid cells by combined immunofluorescence and phagocytosis assays. *Am J Hum Genet*. 1989; 45: 786–92.
100. **Priest RE, Marimuthu KM, Priest JH.** Origin of cells in human amniotic fluid cultures: ultrastructural features. *Lab Invest*. 1978; 39: 106–9.
101. **De Coppi P, Bartsch G Jr, Siddiqui MM, *et al.*** Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol*. 2007; 25: 100–6.
102. **Sarić T, Frenzel LP, Hescheler J.** Immunological barriers to embryonic stem cell-derived therapies. *Cells Tissues Organs*. 2008; 188: 78–90.
103. **Daley GQ, Scadden DT.** Prospects for stem cell-based therapy. *Cell*. 2008; 132: 544–8.
104. **Deb KD, Sarda K.** Human embryonic stem cells: preclinical perspectives. *J Transl Med*. 2008; 6: 7.
105. **Carlsson PO, Mattsson G.** Oxygen tension and blood flow in relation to revascularization in transplanted adult and fetal rat pancreatic islets. *Cell Transplant*. 2002; 11: 813–20.
106. **Roh JK, Jung KH, Chu K.** Adult stem cell transplantation in stroke: its limitations and prospects. *Curr Stem Cell Res Ther*. 2008; 3: 185–96.
107. **Hwang WS, Lee BC, Lee CK, *et al.*** Cloned human embryonic stem cells for tissue repair and transplantation. *Stem Cell Rev*. 2005; 1: 99–109.
108. **Hwang WS, Roh SI, Lee BC, *et al.*** Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science*. 2005; 308: 1777–83.
109. **Hwang WS, Ryu YJ, Park JH, *et al.*** Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science*. 2004; 303: 1669–74.
110. **Kennedy D.** Editorial retraction. *Science*. 2006; 311: 335.
111. **French AJ, Adams CA, Anderson LS, *et al.*** Development of human cloned blastocysts following somatic cell nuclear transfer (SCNT) with adult fibroblasts. *Stem Cells*. 2008; 26: 485–93.
112. **Collas P.** Nuclear reprogramming in cell-free extracts. *Philos Trans R Soc Lond B Biol Sci*. 2003; 358: 1389–95.
113. **Collas P, Taranger CK.** Epigenetic reprogramming of nuclei using cell extracts. *Stem Cell Rev*. 2006; 2: 309–17.
114. **Collas P, Taranger CK.** Toward reprogramming cells to pluripotency. *Ernst Schering Res Found Workshop*. 2006; 47–67.
115. **Collas P, Taranger CK, Boquest AC, *et al.*** On the way to reprogramming cells to pluripotency using cell-free extracts. *Reprod Biomed Online*. 2006; 12: 762–70.
116. **Cowan CA, Atienza J, Melton DA, *et al.*** Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science*. 2005; 309: 1369–73.
117. **Takahashi K, Yamanaka S.** Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126: 663–76.
118. **Takahashi K, Tanabe K, Ohnuki M, *et al.*** Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131: 861–72.
119. **Yu J, Vodyanik MA, Smuga-Otto K, *et al.*** Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318: 1917–20.
120. **Hanna J, Wernig M, Markoulaki S, *et al.*** Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science*. 2007; 318: 1920–3.
121. **Okita K, Ichisaka T, Yamanaka S.** Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007; 448: 313–7.
122. **Okita K, Nakagawa M, Hyenjong H, *et al.*** Generation of mouse induced pluripotent stem cells without viral vectors. *Science*. 2008; 322: 949–53.
123. **Stadtfeld M, Nagaya M, Utikal J, *et al.*** Induced pluripotent stem cells generated without viral integration. *Science*. 2008; 322: 945–9.
124. **Moore KE, Mills JF, Thornton MM.** Alternative sources of adult stem cells: a possible solution to the embryonic stem cell debate. *Gend Med*. 2006; 3: 161–8.
125. **Bradley JA, Bolton EM, Pedersen RA.** Stem cell medicine encounters the immune system. *Nat Rev Immunol*. 2002; 2: 859–71.
126. **Drukker M, Katz G, Urbach A, *et al.*** Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc Natl Acad Sci USA*. 2002; 99: 9864–9.
127. **Draper JS, Pigott C, Thomson JA, Andrews PW.** Surface antigens of human embryonic stem cells: changes upon differentiation in culture. *J Anat*. 2002; 200: 249–58.
128. **Drukker M, Katchman H, Katz G, *et al.*** Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells*. 2006; 24: 221–9.
129. **Grinnemo KH, Kumagai-Braesch M, Mansson-Broberg A, *et al.*** Human embryonic stem cells are immunogenic in allogeneic and xenogeneic settings. *Reprod Biomed Online*. 2006; 13: 712–24.
130. **Simpson E, Scott D, James E, *et al.*** Minor H antigens: genes and peptides. *Eur J Immunogenet*. 2001; 28: 505–13.
131. **Cooper DK.** Clinical survey of heart transplantation between ABO blood group-incompatible recipients and donors. *J Heart Transplant*. 1990; 9: 376–81.
132. **Atoui R, Asenjo JF, Duong M, *et al.*** Marrow stromal cells as universal donor cells for myocardial regenerative therapy: their unique immune tolerance. *Ann Thorac Surg*. 2008; 85: 571–9.
133. **Gajewski JL, Johnson VV, Sandler SG, *et al.*** A review of transfusion practice before, during, and after hematopoietic progenitor cell transplantation. *Blood*. 2008; 112: 3036–47.
134. **Appleman LJ, Boussiotis VA.** T cell anergy and costimulation. *Immunol Rev*. 2003; 192: 161–80.
135. **Dugast AS, Vanhove B.** Immune regulation by non-lymphoid cells in transplantation. *Clin Exp Immunol*. 2009; 156: 25–34.
136. **Haeryfar SM, Lan Z, Leon-Ponte M, *et al.*** Prolongation of cardiac allograft survival by rapamycin and the invariant natural killer T cell glycolipid agonist OCH. *Transplantation*. 2008; 86: 460–8.
137. **Yu J, Venstrom JM, Liu XR, *et al.*** Breaking tolerance to self, circulating natural killer cells expressing inhibitory KIR for non-self HLA exhibit effector function following T-cell depleted allogeneic hematopoietic cell transplantation. *Blood*. 2009; 113: 3875–84.
138. **Wakao H, Wakao R, Sakata S, *et al.*** *In vitro* induction of natural killer T cells from embryonic stem cells prepared using somatic cell nuclear transfer. *FASEB J*. 2008; 22: 2223–31.
139. **Miki T, Lehmann T, Cai H, *et al.*** Stem cell characteristics of amniotic epithelial cells. *Stem Cells*. 2005; 23: 1549–59.
140. **Miki T, Strom SC.** Amnion-derived pluripotent/multipotent stem cells. *Stem Cell Rev*. 2006; 2: 133–42.
141. **Horwitz EM, Gordon PL, Koo WK, *et al.*** Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc Natl Acad Sci USA*. 2002; 99: 8932–7.
142. **Di Nicola M, Carlo-Stella C, Magni M, *et al.*** Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood*. 2002; 99: 3838–43.
143. **Romieu-Mourez R, Francois M, Boivin MN, *et al.*** Regulation of MHC class II expression and antigen processing in murine and human mesenchymal stromal

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd

cells by IFN-gamma, TGF-beta, and cell density. *J Immunol.* 2007; 179: 1549–58.
144. **Morelli AE, Thomson AW.** Dendritic cells: regulators of alloimmunity and opportunities for tolerance induction. *Immunol Rev.* 2003; 196: 125–46.
145. **Jiang XX, Zhang Y, Liu B, *et al.*** Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood.* 2005; 105: 4120–6.
146. **Brusko TM, Putnam AL, Bluestone JA.** Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. *Immunol Rev.* 2008; 223: 371–90.
147. **Degauque N, Mariat C, Kenny J, *et al.*** Immunostimulatory Tim-1-specific antibody deprograms Tregs and prevents transplant tolerance in mice. *J Clin Invest.* 2008; 118: 735–41.
148. **Hince M, Sakkal S, Vlahos K, *et al.*** The role of sex steroids and gonadectomy in the control of thymic involution. *Cell Immunol.* 2008; 252: 122–38.
149. **Ildstad ST, Sachs DH.** Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature.* 1984; 307: 168–70.
150. **Ildstad ST, Wren SM, Bluestone JA, *et al.*** Characterization of mixed allogeneic chimeras. Immunocompetence, *in vitro* reactivity, and genetic specificity of tolerance. *J Exp Med.* 1985; 162: 231–44.
151. **Kawai T, Cosimi AB, Spitzer TR, *et al.*** HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med.* 2008; 358: 353–61.
152. **Alpdogan O, Schmaltz C, Muriglan SJ, *et al.*** Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-versus-host disease. *Blood.* 2001; 98: 2256–65.
153. **Wils EJ, Braakman E, Verjans GM, *et al.*** Flt3 ligand expands lymphoid progenitors prior to recovery of thymopoiesis and accelerates T cell reconstitution after bone marrow transplantation. *J Immunol.* 2007; 178: 3551–7.
154. **Sutherland JS, Goldberg GL, Hammett MV, *et al.*** Activation of thymic regeneration in mice and humans following androgen blockade. *J Immunol.* 2005; 175: 2741–53.
155. **Heng TS, Goldberg GL, Gray DH, *et al.*** Effects of castration on thymocyte development in two different models of thymic involution. *J Immunol.* 2005; 175: 2982–93.
156. **Fandrich F, Dresske B, Bader M, *et al.*** Embryonic stem cells share immune-privileged features relevant for tolerance induction. *J Mol Med.* 2002; 80: 343–50.
157. **Koch KS, Son KH, Maehr R, *et al.*** Immune-privileged embryonic Swiss mouse STO and STO cell-derived progenitor cells: major histocompatibility complex and cell differentiation antigen expression patterns resemble those of human embryonic stem cell lines. *Immunology.* 2006; 119: 98–115.
158. **Priddle H, Jones DR, Burridge PW, *et al.*** Hematopoiesis from human embryonic stem cells: overcoming the immune barrier in stem cell therapies. *Stem Cells.* 2006; 24: 815–24.
159. **Robertson NJ, Brook FA, Gardner RL, *et al.*** Embryonic stem cell-derived tissues are immunogenic but their inherent immune privilege promotes the induction of tolerance. *Proc Natl Acad Sci USA.* 2007; 104: 20920–5.
160. **Wu DC, Boyd AS, Wood KJ.** Embryonic stem cells and their differentiated derivatives have a fragile immune privilege but still represent novel targets of immune attack. *Stem Cells.* 2008; 26: 1939–50.
161. **Ilancheran S, Michalska A, Peh G, *et al.*** Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod.* 2007; 77: 577–88.
162. **Troyer DL, Weiss ML.** Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells.* 2008; 26: 591–9.
163. **Weiss ML, Anderson C, Medicetty S, *et al.*** Immune properties of human umbilical cord Wharton's jelly-derived cells. *Stem Cells.* 2008; 26: 2865–74.
164. **Weiss ML, Medicetty S, Bledsoe AR, *et al.*** Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells.* 2006; 24: 781–92.
165. **Montjovent MO, Bocelli-Tyndall C, Scaletta C, *et al.*** *In vitro* characterization of immune-related properties of human fetal bone cells for potential tissue engineering applications. *Tissue Eng A.* 2009; 15: 1523–32.
166. **Morrow CM, Hostetler CE, Griswold MD, *et al.*** ETV5 is required for continuous spermatogenesis in adult mice and may mediate blood testes barrier function and testicular immune privilege. *Ann N Y Acad Sci.* 2007; 1120: 144–51.
167. **Paus R, Arck P, Tiede S.** (Neuro-) endocrinology of epithelial hair follicle stem cells. *Mol Cell Endocrinol.* 2008; 288: 38–51.
168. **Thomson JA, Odorico JS.** Human embryonic stem cell and embryonic germ cell lines. *Trends Biotechnol.* 2000; 18: 53–7.
169. **Amit M, Itskovitz-Eldor J.** Derivation and spontaneous differentiation of human embryonic stem cells. *J Anat.* 2002; 200: 225–32.
170. **Kastenberg ZJ, Odorico JS.** Alternative sources of pluripotency: science, ethics, and stem cells. *Transplant Rev.* 2008; 22: 215–22.
171. **Amit M, Shariki C, Margulets V, *et al.*** Feeder layer- and serum-free culture of human embryonic stem cells. *Biol Reprod.* 2004; 70: 837–45.
172. **Xu C, Inokuma MS, Denham J, *et al.*** Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol.* 2001; 19: 971–4.
173. **Klimanskaya I, Chung Y, Meisner L, *et al.*** Human embryonic stem cells derived without feeder cells. *Lancet.* 2005; 365: 1636–41.
174. **Allegrucci C, Wu YZ, Thurston A, *et al.*** Restriction landmark genome scanning identifies culture-induced DNA methylation instability in the human embryonic stem cell epigenome. *Hum Mol Genet.* 2007; 16: 1253–68.
175. **Condic ML, Rao M.** Regulatory issues for personalized pluripotent cells. *Stem Cells.* 2008; 26: 2753–8.
176. **Le Blanc K, Samuelsson H, Gustafsson B, *et al.*** Transplantation of mesenchymal stem cells to enhance engraftment of hematopoietic stem cells. *Leukemia.* 2007; 21: 1733–8.
177. **Roy NS, Nakano T, Keyoung HM, *et al.*** Telomerase immortalization of neuronally restricted progenitor cells derived from the human fetal spinal cord. *Nat Biotechnol.* 2004; 22: 297–305.
178. **Smith SB, Gasa R, Watada H, *et al.*** Neurogenin3 and hepatic nuclear factor 1 cooperate in activating pancreatic expression of Pax4. *J Biol Chem.* 2003; 278: 38254–9.
179. **Vassilopoulos G, Wang PR, Russell DW.** Transplanted bone marrow regenerates liver by cell fusion. *Nature.* 2003; 422: 901–4.
180. **Rizzino A.** A challenge for regenerative medicine: proper genetic programming, not cellular mimicry. *Dev Dyn.* 2007; 236: 3199–207.
181. **Clarke DL, Johansson CB, Wilbertz J, *et al.*** Generalized potential of adult neural stem cells. *Science.* 2000; 288: 1660–3.
182. **D'Amour KA, Gage FH.** Genetic and functional differences between multipotent neural and pluripotent embryonic stem cells. *Proc Natl Acad Sci USA.* 2003; 100: 11866–72.

© 2009 The Authors
Journal compilation © 2009 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd