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Immunogenicity of Stem Cells and Their Derivatives

Imunogenicita kmenových buněk a jejich derivátů

Bachelor thesis

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Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstract

Stem cells (SCs) have the potential to be used in regenerative medicine on the basis of their differentiation capacity and promising immunological properties, including low expression of histocompatibility antigens and costimulatory molecules, or secretion of suppressive cytokines. Their immunogenicity has often been ignored in the past but it is becoming clear that rejection of genetically incompatible SCs represents a very common issue. At present, SCs are extensively studied from the immunological point of view, since it represents a critical aspect of the safety of SC therapy. This thesis presents an overview of current knowledge about immunogenicity of SCs and their derivatives, including both pluripotent SCs (embryonic and induced pluripotent SCs) and adult SCs (mesenchymal, limbal, neural, haematopoietic and umbilical cord blood SCs). The expression of immunologically relevant molecules on their surface and interaction with the immune cells *in vitro* and *in vivo* will be discussed, together with suggestions for overcoming the immunological barriers for transplantation. Detailed analysis of these aspects necessarily has to precede the safe clinical translation of SC therapies.

Keywords

Stem cells, stem cell differentiation, immunogenicity, membrane antigens, regenerative medicine.

Abstrakt

Kmenové buňky mají potenciál v regenerativní medicíně především z důvodu své schopnosti diferenciovat v řadu buněčných linií a také nízké imunogenicity. Ta vyplývá mimo jiné z nízké exprese histokompatibilních antigenů a kostimulačních molekul nebo sekrece tlumivých cytokinů. Imunogenicita tkání odvozených z kmenových buněk byla v minulosti často přehlížena, ale dnes je zřejmé, že rejekce představuje nezanedbatelné riziko při jejich transplantaci. Jejich imunologickým vlastnostem je tedy věnována velká pozornost, aby bylo možné zaručit bezpečnost léčby kmenovými buňkami. Tato bakalářská práce se snaží nastínit současné poznatky o imunogenicitě kmenových buněk a jejich derivátů – zmíněny jsou jak pluripotentní kmenové buňky (embryonální a indukované pluripotentní), tak dospělé kmenové buňky (mezenchymální, limbální, neurální, hematopoetické a kmenové buňky z pupečníkové krve). Exprese imunologicky významných molekul a interakce s buňkami imunitního systému *in vitro* a *in vivo* tvoří v této práci základ pro hodnocení imunogenicity. Společně s těmito vlastnostmi budou prezentovány možné způsoby překonání imunologických bariér při transplantaci kmenových buněk. Teprve po pečlivé analýze všech těchto faktorů bude možné poznatky o kmenových buňkách bezpečně využít v klinické praxi.

Klíčová slova

Kmenové buňky, diferenciace kmenových buněk, imunogenicita, membránové antigeny, regenerativní medicína.

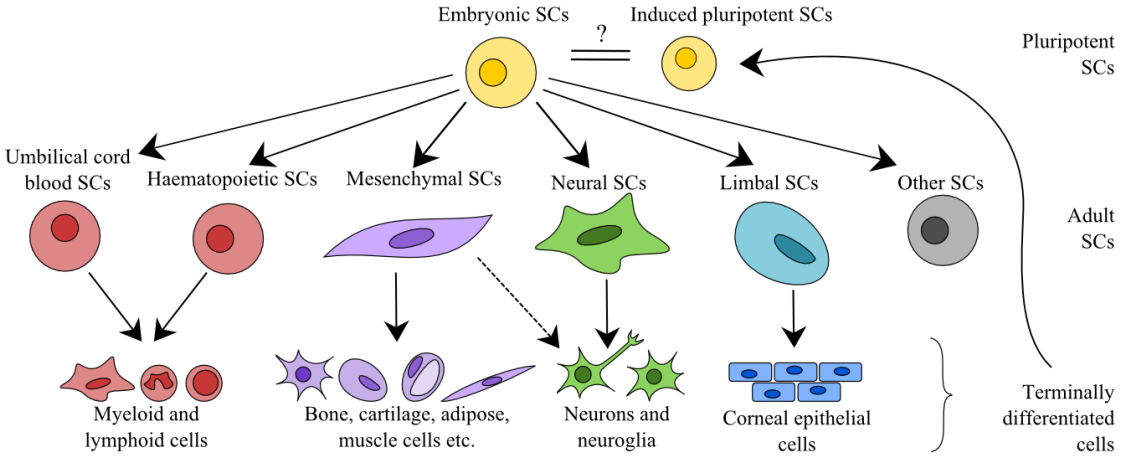
Content

Abbreviations

APCs	antigen presenting cells
DCs	dendritic cells
EB	embryoid body
ESCs	embryonic stem cells
FasL	Fas ligand
GvHD	graft-versus-host disease
hESCs	human embryonic stem cells
HLA	human leukocyte antigen
HSCs	hematopoietic stem cells
ICAM-1	intercellular adhesion molecule 1
IDO	indoleamine-2,3-dioxygenase
IFN	interferon
IL	interleukin
iPSCs	induced pluripotent stem cells
LSCs	limbal stem cells
mESCs	murine embryonic stem cells
MHC	major histocompatibility complex
MSCs	mesenchymal stem cells
NK	natural killer
PD-L1	programmed death-ligand 1
PGE2	prostaglandin E2
SCs	stem cells
TGF-β	transforming growth factor β
TLR	Toll-like receptor
TNF-α	tumour necrosis factor α
Tregs	regulatory T cells
UCB SCs	umbilical cord blood stem cells

1 Introduction

The diverse repertoire of stem cells (SCs) is defined by two fundamental characteristics – the ability of self-renewal and the capacity to differentiate into many cell lineages. Pluripotent SCs, namely embryonic stem cells (ESCs) and their artificial counterpart – induced pluripotent stem cells (iPSCs) – are capable of giving rise to cells of all three germ layers. In somatic tissues, the multipotent cells securing physiological regeneration are referred to as adult SCs and serve as progenitors of terminally differentiated cells (Figure 1). SCs hold the potential for a treatment of various diseases but such therapies face number of issues, including immunogenicity, which complicates their engraftment. Conversely, some SCs can act as efficient suppressors of inflammation. These facts highlight the necessity of studying their immunological properties.



ESCs are pluripotent derivatives of the inner cell mass of the blastocyst. They manifest an extensive differentiation capacity and were shown to be less immunogenic than adult body cells. However, there are justified concerns about teratoma formation after transplantation and a danger of abusing the immune privilege by viral infections or cancer. Moreover, ESCs face important ethical questions regarding the use of cells derived from human embryos.

Artificial induction of pluripotency in adult cells, first introduced by Takahashi and Yamanaka in 2006, offered a new hope for SC therapies. Since the reprogrammed cells would share the same genetic background with patient's cells, iPSCs were expected to be non-immunogenic. However, some doubts have arisen about this assumption and to ensure their immune privilege, it will be necessary to further define these cells and design

safer ways of reprogramming. Teratoma formation, malignant transformation and expression of novel markers represent the greatest concerns for iPSCs.

Adult SCs are multipotent and unipotent progenitors of terminally differentiated cell lines. They have been found in majority of body tissues. Clinically significant types of multipotent cells are mesenchymal stem/stromal cells (MSCs), limbal stem cells (LSCs), neural stem cells (NSCs), haematopoietic stem cells (HSCs) or their less mature alternative – umbilical cord blood stem cells (UCB SCs) and others.

The most extensively studied MSCs give rise to a wide range of cells, including bone, cartilage, muscle, adipose or even neural cells. They play a crucial role in tissue homeostasis – they have the ability to migrate into the site of damage and to produce a variety of suppressive molecules which can inhibit immune responses. However, their advantageous properties can be lost and MSCs can contribute to inflammatory processes.

HSCs are progenitors of all lymphoid and myeloid cells which together form the immune system of an organism. They are widely used for treatment of various haematological malignancies such as leukaemia. Recently, it was shown that their immune privilege is not only a result of their residence in the HSC niche but they are also endowed with suppressive abilities. However, outside of the niche, they can become more immunogenic. In case that it is not possible to find a suitable donor of HSCs, UCB SCs represent a good alternative. They seem to be less differentiated and therefore might be slightly advantaged for engraftment.

Similarly, other adult SCs own potential in regenerative medicine. NSCs give rise to both neurons and neuroglia and therefore might serve as a treatment for many neurological disorders. LSCs secure the regeneration of cornea. Both of these cells were shown to have immunosuppressive properties but also to change their characteristics when manipulated or exposed to stress conditions. There are many more stem and progenitor cells in the body, literally in every tissue. However, not all of them were studied for their immunological characteristics and will not be included in this thesis.

All the above mentioned SCs obviously own some advantageous characteristics which offer an opportunity to discover novel therapies for diseases when handled appropriately. All of them also face the risk of rejection when transplanted into genetically incompatible recipient. The task is now to examine these characteristics further and design ways to successfully and safely translate the findings for clinical use.

2 Aims of the thesis

The aim of this thesis is to present a concise overview about the recent knowledge of immunogenicity and immune privilege of pluripotent and adult SCs, including their derivatives, and to draw some analogies valid for immune response to these cells. It will consist of summary of research papers dealing with expression of immunologically relevant molecules and interaction of SCs with the immune cells *in vitro* and *in vivo*. The thesis will focus on ESCs, iPSCs and MSCs in more detail, as they have been most extensively studied. Next, the immunological properties of all included SCs will be compared. The advantages and disadvantages of their use in the clinic and the ways how to overcome the immunological complications will be shortly discussed. Finally, an outline of future directions in research regarding this field will be presented.

3 Pluripotent stem cells

3.1 Embryonic stem cells

ESCs were originally thought to own immunoprivileged phenotype due to limited expression of major histocompatibility complex (MHC). Molecules of MHC class I and II were not found on mouse ESCs (mESCs) (Tian et al., 1997) and later studies generally agree with these findings. A study performed with rat ESCs showed that MHC I molecules are weakly expressed and MHC II protein was not detected at all (Fändrich et al., 2002). Very low expression of MHC I and absence of MHC II molecule was observed also on undifferentiated human ESCs (hESCs) (Drukker et al., 2002), and is possibly regulated epigenetically (Suárez-Álvarez et al., 2010).

Expression of MHC molecule can be induced by extrinsic factors, primarily by interferons (IFNs). IFN-α and IFN-β did not change the expression on MHC I protein in ESCs (Drukker et al., 2002), whereas IFN-γ provides more powerful stimulus, correlating with the expression of IFN receptors. Although Tian et al. (1997) showed that MHC I and II molecules on the surface of undifferentiated ESCs could not be induced by treatment with IFNγ, most of the later studies suggest that adding IFN-γ to the culture causes up-regulation of the MHC I but not MHC II molecules (Drukker et al., 2002). The effects of interferons highlight the importance of not ignoring the capacity of cytokines to change the characteristics of ESCs because they can be secreted *in vivo* and modify the immune privilege of ESCs.

Since ESCs have only low expression of MHC I molecule, they represent a potential target for NK (natural killer) cells. However, ligands for activating NK receptor NKG2D were weakly expressed on human (Suárez-Álvarez et al., 2010) and murine ESCs (Dressel et al., 2008). Additionally, the expression of ICAM-1 (intercellular adhesion molecule 1) was elevated on undifferentiated mESCs, but decreased after differentiation (Tian et al., 1997). Interaction of ICAM-1 with LFA-1 (lymphocyte function-associated antigen 1) receptor on NK cell could provide a sort of "costimulatory signal" for NK cells. In accordance with this phenotype, it would be expectable that ESCs will be a subject for NK cell-mediated lysis. Nevertheless, Drukker et al. (2002) showed that ESCs are not efficiently targeted by NKs. It seems that NK cells require to be previously activated to target ESCs. However, there is not a consensus about this: Frenzel et al. (2009) showed that undifferentiated mESCs are effectively lysed by NK cells even

without their activation, whereas Koch et al. (2008) did not detect mESC lysis even by pre-activated NK cells.

Recognition of an antigen on MHC molecule by immune cells is not sufficient to provoke immune response, since they require additional signals. No expression of costimulatory molecules CD80 and CD86 was found on rat ESCs (Fändrich et al., 2002). Human ESCs also seem to be negative for CD80 and CD86 (Drukker et al., 2006) and even for CD40 expression. (Li et al., 2004). This is not surprising, considering that the costimulatory molecules are usually expressed predominantly by antigen presenting cells (APCs). However, in case of differentiation into an APC, or an endothelial cell, they might appear on the cell surface and assist with the graft rejection.

Non-classical MHC I molecules, such as HLA-E (human leukocyte antigen E) or HLA-G could potentially contribute to the immune privilege – they are associated with protection of the foetus during pregnancy. Although they were originally not found to be expressed on hESCs (Drukker et al., 2002), other groups were able to detect HLA-G mRNA (Grinnemo et al., 2006) and even the final protein on several hESC lines (Verloes et al., 2011).

Ligands for activating NK receptors in human – MICA, MICB and ULBP1, 2 and 3 – are not expressed on the cell surface of hESCs, although probably regulated post-transcriptionally because mRNA for MICA and MICB were detected (Suárez-Álvarez et al., 2010). In mouse, these ligands include RAE-1, H60, and MULT-1 and apart from RAE-1, they were not found on undifferentiated mESCs (Bonde & Zavazava, 2006; Dressel et al., 2008).

Additionally, higher expression of Fas ligand (FasL) could be partly responsible for the impaired ability of the immune system to reject ESCs. FasL-positive ESCs could induce the apoptosis of activated lymphocytes and thus prevent the rejection. Expression of FasL on ESCs has been shown in rat (Fändrich et al., 2002) and mouse (Bonde & Zavazava, 2006). For hESCs, increased expression of FasL was not confirmed (Drukker et al., 2006; Grinnemo et al., 2006).

Moreover, soluble factors secreted by ESCs or their derivatives could create an immunosuppressive environment in the site of transplant. There is not an agreement about which molecules ESCs secrete. One group could not detect the expression of anti-inflammatory cytokines transforming growth factor β (TGF-β) or interleukin 10 (IL-10)

(Grinnemo et al., 2006) whereas others showed that expression of TGF-β is elevated in undifferentiated hESCs (Koch et al., 2008). Also the elevated production of arginase I, an enzyme degrading L-arginine which can cause anergy in T cells, was confirmed for hESCs (Yachimovich-Cohen et al., 2010).

The knowledge of expressed molecules is only sufficient to draw theoretical conclusions. To judge the real immunogenicity of ESCs, the reaction of the immune cells, primarily of the T cells, needs to be assayed *in vitro* and *in vivo*. ESCs were not shown to cause proliferation of T cells in co-culture and might even own some immunosuppressive properties since they even suppressed the proliferation of T lymphocytes which were already activated by contact with allogenic dendritic cells (DCs). Whether this is mediated via direct membrane contact (Li et al., 2004) or soluble factors (Koch et al., 2008), remains to be answered. Another considered mechanism of suppression is induction of regulatory T cells (Tregs) but this has not been confirmed (Koch et al., 2008).

Despite of these promising data from *in vitro* experiments, complex cellular and humoral response against hESC graft could be measured five days after injection in mouse model (Grinnemo et al., 2006). Accumulation of T cells around the graft was observed in the *in vivo* mouse model of myocardial ischemia several weeks after injection of undifferentiated allogeneic mESCs (Kofidis et al., 2005). Moreover, ongoing differentiation was connected with increasing T cell infiltration and subsequently the allogeneic graft was eliminated completely (Swijnenburg et al., 2005). However, immune response in these studies might have been partly set off by the danger signals from damaged ischemic tissue, or by genetic modification of ESCs.

Although ESCs could be used for induction of immune tolerance or in tissue regeneration also without previous differentiation (Koch et al., 2008), in most therapies, pluripotent cells will need to be differentiated to a specific tissue and purified before transplantation to the recipient in order to prevent formation of teratomas. After spontaneous differentiation of ESCs into embryoid bodies (EBs) *in vitro*, the expression of MHC I and II molecules remains undetectable in mouse model (Tian et al., 1997). Conversely, after *in vivo* differentiation into teratomas, the expression of MHC molecules seems to increase, suggesting that the effects of the microenvironment are crucial for the development of the mESCs (Kofidis et al., 2005). The differentiation of human ESCs *in vitro* or *in vivo* leads to small increase of MHC I molecules but in both cases it still remains below the level in differentiated somatic cells. Expression of MHC II protein

(Drukker et al., 2002) and costimulatory molecules (Li et al., 2004) was not detected in ESCs derivatives. ESC-derived cells seem to be able to repulse the NK cell attack (Drukker et al., 2002; Frenzel et al., 2009). Whether it is due to inhibition of NK cells by the increased expression of MHC I molecule or other mechanism is involved, remains to be discovered.

ESC-derived cells were studied in animal models. Hematopoietic derivatives were not rejected and did not develop graft-versus-host disease (GvHD) in a mismatched recipient mouse (Burt et al., 2004). Derived neural cells were also not rejected and showed a capability of improvement of mobility after spinal cord injury (McDonald et al., 1999). On a contrary, insulin producing cell clusters derived from mESCs were rapidly rejected when transplanted to both allogeneic and syngeneic recipients, probably due to expression of novel markers (Wu et al., 2008). These and many more studies provide encouraging data but also evidences that the immune privilege of ESCs is not absolute.

In conclusion, most of the studies agree that the MHC I molecule expression on ESCs from different model animals or human is lower than in differentiated somatic cells and MHC II molecule is absent completely. Nevertheless, expression of histocompatibility antigens can be enhanced by certain cytokines or by differentiation. Although ESCs and their derivatives might seem immunoprivileged *in vitro*, the experiments *in vivo* often result in rejection. Due to these signs of immunogenicity, it will probably be necessary to use immunosuppressive drugs to secure their engraftment.

3.2 Induced pluripotent stem cells

The expression of immunologically relevant molecules in iPSCs was shown to be very similar to ESCs by most of the studies. Murine iPSCs were shown to be negative for MHC I molecule (Dressel et al., 2010). In the porcine model, which more closely resembles the human physiology, low amount of MHC I and no MHC II molecules could be detected (Park et al., 2013). In human iPSCs, the levels of MHC I and MHC II molecules were reduced compared to parental fibroblasts (Suárez-Álvarez et al., 2010). Similarly to ESCs, the expression of MHC I protein is increased after addition of IFN-γ to the culture and MHC II molecule remains unaffected. The expression of costimulatory molecules was not detected (Lu et al., 2014). HLA-E in iPSCs was shown to be decreased compared to parental fibroblasts and HLA-G was not found at all (Suárez-Álvarez et al., 2010). The secreted molecules were not defined yet for iPSCs.

In vitro, mouse iPSCs were shown to be efficiently targeted by activated autologous NK cells, and expressing some of the ligands of activating NK receptor NKG2D both in mouse (Dressel et al., 2010) and human (Suárez-Álvarez et al., 2010). *In vivo*, NK cells must also be pre-activated to efficiently prevent engraftment of iPSCs (Dressel et al., 2010). Regarding T cell response, undifferentiated iPSCs were not targeted by syngeneic T cells *in vitro* (Guha et al., 2013). They also did not induce the expression of activation markers on T cells. However, they caused proliferation of allogeneic CD4+ T cells. Moreover, they induce the *in situ* T cells to secrete IL-2 and IL-10, and contribute to induction of Tregs (Lu et al., 2014).

It was shown that the human immune system includes memory T cells reactive against the crucial pluripotency antigen Oct4 which could possibly cause immune response against iPSCs (Dhodapkar et al., 2010). Another novel markers can be introduced during generation of iPSCs using both retroviral and episomal vectors, such as tumour antigen Hormad1 or tissue-specific antigen Spt1. This can result in T cell infiltration of formed teratomas in syngeneic mice, which rarely happens in teratomas derived from ESCs (Zhao et al., 2011). Araki et al. (2013) did not confirm these results for various iPSC lines as the teratomas all manifested very low immunogenicity.

In vitro differentiated iPSCs were shown to express low level of MHC I molecules and costimulatory molecules CD80, CD86 and CD40, and therefore were hypothetically capable of provoking immune response. This was demonstrated by increased proliferation of allogeneic T cells in co-culture with iPSCs (Guha et al., 2013). Upon differentiation, the level of MHC I molecules was slightly increased in porcine model, but MHC II molecules remained undetectable (Park et al., 2013). Levels of MHC molecules on iPSC-derived haematopoietic progenitors were shown to be lower than in control fibroblasts and they did not express costimulatory molecules. Additionally, low levels of HLA-E and HLA-G were found on their surface (Kim et al., 2013).

Regarding *in vivo* experiments, Araki et al. (2013) reported successful transplantation of terminally differentiated skin and bone marrow derivatives from iPSCs and ESCs. However, unlike these cells which were differentiated *in vivo* in chimeric mouse, the *in vitro* differentiated cardiomyocytes were shown to be much more immunogenic. However, Guha et al. (2013) transplanted the *in vitro* differentiated hepatocytes, endothelial cells and neurons almost devoid of immunogenicity. To provide a more clinically relevant data, Morizane et al. (2013) used a nonhuman primate model

for demonstration of immune privilege of autologous derivatives of iPSCs. These authors also further highlighted that viral transduction of the cells is a less safe method of inducting pluripotency regarding the immunogenicity of the final product.

A plenty of reprogramming methods have been developed and some of them were found to give rise to more immunogenic cells. It is therefore necessary to take these aspects into consideration when aiming for the safest possible source of iPSCs. Additionally, during reprogramming of iPSCs, the cells tend to "remember" their origin by increased expression of markers of the cells they were differentiated from. Polo et al. (2010) showed that iPSCs from different sources were shown to be distinguishable by their epigenetic profile and subsequently gene expression. These differences could also affect some of the molecules mentioned earlier and therefore have impact on the immunogenicity.

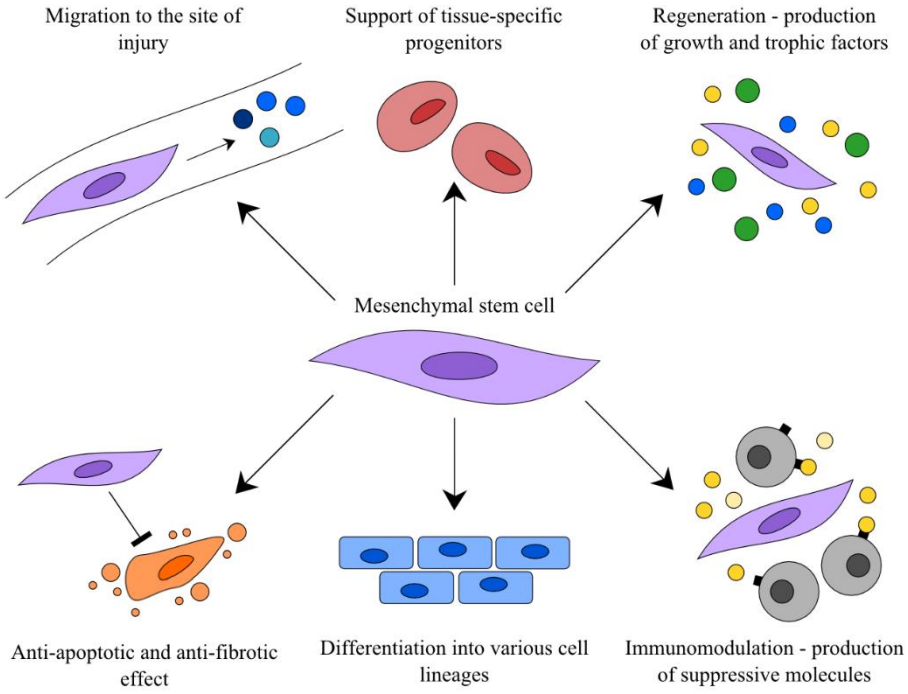
To summarise, iPSCs resemble ESCs in many properties, as expected. They express low MHC I and lack MHC II molecules and costimulatory molecules. The difference can only be found in the expression of HLA-G molecule, which is most likely expressed by ESCs but not by iPSCs. Next, both are targeted by activated NK cells and seem to be immune to T cell attack *in vitro*. However, especially when differentiated *in vitro*, iPSCs derivatives seem to gain stronger immunogenicity. For this reason and due to lack of clarity regarding the reprogramming methods safety, the iPSCs therapies will probably be developed later then it was initially hoped for.

4 Adult stem cells

4.1 Mesenchymal stem cells

MSCs hold immense therapeutic potential on account of combination of qualities which help to regenerate damaged body tissues. Among these qualities, which are summarized in Figure 2, immunomodulation plays an important role.

Figure 2. Therapeutic effects of MSCs. (redrawn from Holan & Javorkova, 2013)



Initially, MSCs were thought to own a considerable immune privilege thanks to their demonstrated immunosuppressive properties. It partially lies in expression of surface molecules. MSCs and derived bone, cartilage and adipose tissue express intermediate level of MHC class I molecule but MHC class II molecule was only detected after IFN-γ treatment of undifferentiated MSCs (Le Blanc et al., 2003). Additionally, MSCs lack costimulatory molecules CD80, CD86 and CD40 or adhesion molecule ICAM-1 (Tse et al., 2003). Alongside, they express other molecules which contribute to their immune privilege, such as HLA-G (Nasef et al., 2007), programmed death-ligand 1 (PD-L1) and FasL (Gu et al., 2013). However, the physical contact with immune cells was shown as supportive but dispensable for the immunosuppression by MSCs (Di Nicola et al., 2002).

The seemingly predominant mechanisms which MSCs use to inhibit immune response, is secretion of suppressive cytokines and chemokines. Immune cells are attracted to MSCs and subsequently suppressed by secreted factors. Both murine and human MSCs secrete prostaglandin E2 (PGE2), IL-6 and heme oxygenase-1 (HO-1) and others. The role of IL-10, which is usually detected in MSC cultures and *in vivo* MSC grafts, needs to be further investigated since it is still not clear whether it is produced directly by MSCs or it is the case that MSCs stimulate neighbour cells to secrete it (reviewed in Ma et al., 2014). One of the suggested mechanisms of suppression by murine MSCs is production of nitric oxide by inducible nitric oxide synthase (iNOS) (Sato et al., 2007). Conversely, human MSCs after stimulation with IFN-γ express indoleamine-2,3-dioxygenase (IDO) which secures elimination of tryptophan from the environment, setting a metabolic barrier for immune cells (Meisel et al., 2004).

In agreement with the previously described phenotype, MSCs and their derivatives were shown to have suppressive effect on many immune cell types (reviewed in Ma et al., 2014). Overall, the findings about immunological properties are very encouraging and could be used for treatment of various diseases. *In vivo* experiments further showed that MSCs can efficiently improve healing process in different animal models of diseases or injuries. Donor-derived MSCs contribute to longer survival of graft in allogeneic heart (Popp et al., 2008) or skin (Bartholomew et al., 2002) transplantation and can attenuate GvHD after HSC transplantation (Le Blanc et al., 2004). All of these observations speak in favour of the safe use of MSCs.

Nevertheless, there are conflicting data about the reaction to allogeneic MSCs *in vivo*. As mentioned earlier, many reports state that MSCs support engraftment of allogeneic tissue. However, others show that they are not quite safe because there is a greater probability of eliciting immune response *in vivo* than if autologous MSCs are used (Poncelet et al., 2007). Allogeneic MSCs are eliminated from the recipient earlier than autologous cells (Eliopoulos & Stagg, 2005) or even promote the rejection of transplant when combined with immunosuppressive agent Cyclosporine A (Inoue et al., 2006). To date, there is still a discussion about safety of allogeneic MSCs and these cells should therefore not account for "universal donor" cells.

Even though MSCs can limit the action of immune cells, they can still be eliminated from the body soon after administration (Popp et al., 2008). Nevertheless, their immunosuppressive effect could still be preserved even after their disappearance for

example by induction of Tregs (Maccario et al., 2005) which then reduce the differentiation of effector T cells. Simultaneous immunosuppressive treatment (although in low levels) will probably be necessary to achieve the best results of tolerance induction (Popp et al., 2008).

Moreover, the ability of MSCs to suppress immune cells is probably not evoked without previous activation of the MSCs, i.e. licensing. Such licensing can be performed by variety of cytokines, primarily IFN-γ supplemented with tumour necrosis factor α (TNF-α), IL-1α or IL-1β (Ren et al., 2008). Although high levels of these inflammatory cytokines entitle the MSCs for immune suppression, low levels of inflammation have been shown to elicit an inverse effect. In low concentrations of inflammatory cytokines, MSCs can become efficient presenters of antigen by increased expression of MHC II molecule (Chan et al., 2006) and are even capable of cross-presentation of exogenous antigens on MHC I molecules (Francois et al., 2009). Accordingly, Popp et al. (2008) showed that MSCs only protected the allogeneic heart transplant from acute but not chronic rejection. Similarly, when infusing MSCs before allogeneic transplantation, the graft is rejected more quickly, presumably because the suitable inflammatory environment has not been established yet (Renner et al., 2009).

Waterman et al. (2010) even distinguish the two states as two cell types: pro-inflammatory MS1 type and anti-inflammatory MS2, possibly regulated by stimulation of Toll-like receptors (TLRs) on MSC surface. Specifically, stimulation of TLR3 briefly induces the MS2 phenotype can contribute to navigating the cells towards the damaged tissues and express suppressive IL-4, PGE2 and IDO. When they get to the site of damage, TLR4 ligands provide a stimulus for transformation into MS1 phenotype, capable of immunosuppression in the site of tissue damage by production of pro-inflammatory IL-6, IL-8 and TGF-β.

Manipulation with MSCs could possibly change their immunosuppressive properties or even increase immunogenicity. Cultured MSCs do not share identical characteristics with freshly isolated ones and numerous passaging can lower the production of some cytokines in MSCs. Among other factors which influence the effectivity of the MSC-treatment are the dosage, time and location of the injection or even the stage of the disease (reviewed in Ma et al., 2014).

In conclusion, the phenotype of MSCs helps them to decrease the probability of allogenic recognition and subsequent immune attack. They suppress immune reactions in

both autologous and allogeneic setting mainly by secretion of cytokines. However, their activity is dependent on the environment. Inflammatory conditions most likely activate suppressive activity of MSCs whereas in low or no level of inflammatory cytokines they lose their immune privilege. It is therefore necessary to carefully determine the level of inflammation in the recipient and accordingly administer the therapeutic MSCs at a suitable time (after the development of the disease) and amount.

4.2 Limbal stem cells

LSCs are being successfully used for corneal regeneration but their rejection remains an issue, although they are administered to a presumably immunoprivileged organ.

SCs isolated from the limbus were shown to have rather immunosuppressive than immunogenic properties *in vitro*. Whether the authors talk about limbal MSCs (which supposedly create a niche for LSCs) or LSCs alone, it is clear that cells in the limbus suppress proliferation and cytokine secretion of lymphocytes. The main mechanism of suppression is probably secretion of soluble factors (Holan et al., 2010), including TGFβ1 (Garfias et al., 2012). Suppressive properties were confirmed in mouse (Holan et al., 2010), rabbit (Bray et al., 2014) and human (Garfias et al., 2012). Additionally, limbal MSCs express MHC I molecule but only low level of MHC II molecule which is an analogous phenotype to previously mentioned MSCs (Bray et al., 2014).

Although their immunogenicity alone has not been studied yet, it was demonstrated that freshly isolated LSCs elicit weaker immune response than cryopreserved ones, possibly due to damage during freezing (Zhang et al., 2011).

In conclusion, SCs in the limbus undoubtedly show immunosuppressive characteristics. However, these could be lost during *ex vivo* manipulation or administration and subsequently less effectively support the anti-inflammatory state of the eye.

4.3 Neural stem cells

Neural stem/progenitor cell transplantation has potential to improve the condition of patients with Parkinson's and Huntington's disease or those who have suffered stroke and spinal cord injury. Central nervous system used to be regarded as another immunoprivileged site but lymphocytes can apparently cross the blood-brain barrier and

interact with the transplanted NSCs. Sufficient caution during transplantations should be maintained, which requires perfect knowledge of immunological properties of NSCs.

NSCs show low expression of MHC I and II molecules *in vitro* and *in vivo* (Hori et al., 2003). However, MHC I molecule expression is induced after culture with IFN-γ, IFN-β and TNF-α and MHC II molecule after exposure to IFN-γ (McLaren et al., 2001). Differentiation was shown to increase the expression of MHC I molecule in one study (McLaren et al., 2001) but had no influence in another one (Hori et al., 2003). NSCs also express functional costimulatory molecules CD80 and CD86, expression of which can be enhanced by IFN-γ, and low levels of CD40. CD80 expression was even increased as a reaction to stress conditions (H_2O_2 or serum deprivation) (Imitola et al., 2004). Additionally, FasL was neither transcribed nor translated into protein (Kim et al., 2009).

Undifferentiated and non-stimulated NSCs were shown to slow down proliferation of activated allogeneic lymphocytes *in vitro*, even without direct contact. Interestingly, the expression of IL-4, IL-10, IFN-γ and TNF-α (supposedly in T lymphocytes) was raised and IL-2 decreased during the presence of NSCs in the culture (Kim et al., 2009).

In vivo, NSCs were shown to have some immunosuppressive properties besides their regenerative role in the treatment of diseases (Ben-Hur, 2008). Hori et al. (2003) could observe a partial immune privilege of NSCs but no signs of active suppression of immune reaction. Allogeneic NSCs were shown to survive in a non-immunoprivileged site for at least four weeks without signs of leukocyte infiltration, but they were rejected in a pre-sensitised recipient. Another study showed that NSCs cause T cell infiltration after transplantation to injured brain. Trauma probably caused inflammation in the brain and the secreted cytokines subsequently activated lymphocytes (Zheng et al., 2007).

To summarize, NSCs might have an immunoprivileged phenotype but this only applies for cells which were not exposed to stress or inflammatory cytokines. Because neither NSCs nor central nervous system own an absolute immune privilege, it should be aimed for the best compatibility between the donor and the recipient and additional immunosuppressive treatment should be considered.

4.4 Haematopoietic stem cells

HSCs are currently the only SCs widely used in clinical practice. Bone marrow transplantations (or more appropriately HSC transplantations) require perfect matching of histocompatibility antigens for successful engraftment since HSCs and derived leukocytes naturally express high levels of MHC molecules and other immunologically relevant elements (Lu et al., 2004). They are therefore ideal subjects for allorecognition and effective stimulators of alloresponse.

The immune privilege of undifferentiated HSCs is substantially supported by HSC niche, where they are surrounded by variety of cells, all working together to secure efficient haematopoiesis and HSC self-renewal. Within this niche, HSCs are accompanied by Tregs which can inhibit the possible immune attack by production of IL-10. Allogeneic HSC can survive for a long time if it resides in the HSC niche (Fujisaki et al., 2011).

HSCs were shown to also directly suppress the immune reactions. Stimulated by inflammatory environment, they can enhance their proliferation and differentiation of their progeny into effector cells capable of fighting the infection (Takizawa et al., 2011). Additionally, they express inhibitory receptor CD47 (IAP = integrin-associated protein) to avoid phagocytosis by macrophages (Jaiswal et al., 2009) or PD-L1 to inhibit T cell attack (Zheng et al., 2011). These molecules are upregulated when HSC migrates out from the niche and receives inflammatory or other stress signals.

Overall, undifferentiated HSCs show immunomodulatory properties important for their function, including certain level of immune privilege. However, their indispensable differentiation into blood cells gives rise to fully immunogenic cells. It is also not clear what exactly happens to HSCs when they are outside the niche. Therefore, additional immunosuppression will be necessary to avoid complications following allogeneic transplantations.

4.5 Umbilical cord blood stem cells

UCB SCs account for an alternative of HSCs but their characteristics differ to some extent. Regarding the expression of molecules, UCB SCs lack some of the typical HSC markers and share some characteristics with ESCs, including low expression of MHC I molecules and costimulatory molecules or missing MHC II molecules. They could therefore be seen as less mature version of bone marrow-derived HSCs. Additionally,

they fail to stimulate allogeneic T cell proliferation *in vitro* (Zhao et al., 2006), partially owing to expression of PD-L1 (Zheng et al., 2011).

Transplantations of UCB SCs have been use for treatment of haematological malignancies and the data from the clinic are therefore available for analysis. It was shown that UCB transplantation, when compared to unrelated donor bone marrow transplantation, results in lower risk of GvHD, while their survival rate remains similar. However, the haematopoietic reconstitution requires longer time and is less successful than in bone marrow transplantation (Rocha et al., 2001). Moreover, even mismatched UCB SCs show comparable engraftment success as HLA-matched bone marrow HSCs and matched UCB SCs have better results than all of them (Eapen et al., 2007). UCB SCs therefore represent a valuable alternative in cases where it is not possible to find a suitable bone marrow donor.

5 Discussion

All types of SCs were found to show characteristics which are distinct from fully differentiated somatic cells and at the same time, advantageous for preventing their rejection. They are therefore in the focus of researchers for possible utilisation in therapy. Comparison of expression of immunologically relevant molecules is provided in Table 1. The immature state of SCs is connected with lowered expression of MHC molecules, although inducible by adding IFN-γ to the culture or by differentiation. Lack of costimulatory molecules, expression of HLA-G, FasL and PD-L1, secretion of suppressive cytokines or induction of Tregs are the mechanisms which SCs use to enhance their suppressive qualities. Another advantage of SC transplantation lies in lack of DCs within the graft, a cell population causing severe complications in solid organ transplantation.

Table 1. Expression of immunologically relevant molecules by stem cells.
+ (intermediate or high expression), - (no expression), low (low expression), blank (not defined yet, or the information is not included in this thesis)

	ESCs	iPSCs	MSCs	LSCs	NSCs	HSCs	UCB SCs
MHC I	low	low	+	+	low	+	low
MHC II	-	-	-	low	low	low	-
CD80 CD86	-	-	-		low		
CD40	-	-	-		low		
HLA-G	+	-	+				
FasL	-		+		-		
PD-L1			+		+	+	+

However, none of these mechanisms is capable of ensuring their engraftment without complications. Expression of MHC molecules is decreased but not absent and the expression of suppressive molecules likely has only minor effect. It was shown that SCs are not fully resistant to T cell- or NK cell-mediated cytotoxicity *in vivo*. Predicting immune response against administrated SCs is rather an impossible task because one has to take into account the broad context of the transplantation. This includes individuality of host and donor and anything that could influence their health condition, or a condition of the transplanted cells. It is obvious from the previously mentioned studies that once the SCs are out of their niche, their properties slightly change and can even result in increased immunogenicity.

Many ways of overcoming these immunological barriers have been suggested. Due to expression of MHC molecules and possibly also other histocompatibility antigens, the best possible matching between donor and recipient must be secured. However, the situation could be made easier by creating histocompatibility banks where the most frequent haplotypes of SCs would be stored, ready to be used immediately for treatment. Additional immunosuppression could support the engraftment but their effect can differ from normal organ transplantation and should be tested first. Another approach to achieving tolerance to administered cells would be creation of universal donor cell line, possibly by genetic manipulations. However, cells lacking MHC molecules would be a good target for viruses or malignant transformation and possibly susceptible to NK cell killing. Tolerance to the donor cells could be established by induction of mixed haematopoietic chimerism. To avoid the complications with allogeneicity completely, autologous pluripotent SC lines could be established for individual patients, using either somatic cell nuclear transfer or creating iPSC lines (reviewed in De Rham & Villard, 2011). Nevertheless, these solutions seem rather expensive and time-consuming.

There are many more risks and drawbacks of using all of the SC types than just immunogenicity. For pluripotent cells, it is particularly formation of teratomas, ethical issues and expression of novel antigens. Adult SCs face mostly short supply of the cells and another concern is that they can support the growth of tumours.

Moreover, even though many studies suggest that SC therapies might be safe and successful, long-term effects are unknown. Therefore, in the future, more continuous studies should be done to describe the *in vivo* behaviour of SCs and their therapeutic potential. More attention should also be paid to the immunogenicity of more minor adult SC types which might not be as attractive for studying as pluripotent SCs or MSCs but have considerable potential in regenerative medicine. Last but not least, improvement of the knowledge of the immune system alone is an indispensable prerequisite for drawing correct conclusions about immunogenicity of SCs.

Although SCs might not bring the one-for-all solution in regenerative medicine, as it used to be anticipated, they still represent cells with immense potential, ready to be used to benefit patients. However, with the arising concerns about the immunogenicity of SCs, sufficient caution will be necessary to prevent rejection of the graft or other complications.

6 Conclusion

Discovery of SCs shaped the research objectives of regenerative medicine. Recently, the excitement about SCs subsided and researchers focused on defining the possible risks and ways to overcome them. One of the very painful ones is the immunogenicity, which was not anticipated in the past.

It seems like the capacity of SCs to stimulate immune response is compromised but after transplantation to the challenging *in vivo* environment, they lose the immune privilege. None of the protective mechanisms secures them perfectly from recipient's immune response, particularly when the possibility of differentiation and the influence of present cytokines is regarded. Nevertheless, the immunogenicity of SCs (and their derivatives) is undoubtedly lower than the immunogenicity of somatic cells and could allow to use less aggressive immunosuppressive treatment after transplantation.

Many questions regarding the ability of SCs to provoke immune response remain unanswered. Although some SC types are already in use for treatment of several diseases, defining immunological properties will increase the safety and the efficacy, and possibly allow translation of more therapies for the clinical use.

7 References

Araki, R., Uda, M.M., Hoki, Y., Sunayama, M., Nakamura, M., Ando, S., Sugiura, M., Ideno, H., Shimada, A., Nifuji, A. & Abe, M. (2013). Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature 494*(7435): 100–104.

Bartholomew, A., Sturgeon, C., Siatskas, M., Ferrer, K., McIntosh, K., Patil, S., Hardy, W., Devine, S., Ucker, D., Deans, R., Moseley, A. & Hoffman, R. (2002). Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Experimental Hematology 30*(1): 42–48.

Ben-Hur, T. (2008). Immunomodulation by neural stem cells. *Journal of the Neurological Sciences 265*(1-2): 102–104.

Bonde, S. & Zavazava, N. (2006). Immunogenicity and engraftment of mouse embryonic stem cells in allogeneic recipients. *Stem Cells 24*(10): 2192–2201.

Bray, L.J., Heazlewood, C.F., Munster, D.J., Hutmacher, D.W., Atkinson, K. & Harkin, D.G. (2014). Immunosuppressive properties of mesenchymal stromal cell cultures derived from the limbus of human and rabbit corneas. *Cytotherapy 16*(1): 64–73.

Burt, R.K., Verda, L., Kim, D.-A., Oyama, Y., Luo, K. & Link, C. (2004). Embryonic stem cells as an alternate marrow donor source: engraftment without graft-versus-host disease. *The Journal of Experimental Medicine 199*(7): 895–904.

De Rham, C. & Villard, J. (2011). How to cross immunogenetic hurdles to human embryonic stem cell transplantation. *Seminars in Immunopathology 33*(6): 525–534.

Dhodapkar, K.M., Feldman, D., Matthews, P., Radfar, S., Pickering, R., Turkula, S., Zebroski, H. & Dhodapkar, M. V. (2010). Natural immunity to pluripotency antigen OCT4 in humans. *Proceedings of the National Academy of Sciences of the United States of America 107*(19): 8718–8723.

Di Nicola, M., Carlo-Stella, C., Magni, M., Milanesi, M., Longoni, P.D., Matteucci, P., Grisanti, S. & Gianni, A.M. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood 99*(10): 3838–3843.

Dressel, R., Nolte, J., Elsner, L., Novota, P., Guan, K., Streckfuss-Bömeke, K., Hasenfuss, G., Jaenisch, R. & Engel, W. (2010). Pluripotent stem cells are highly susceptible targets for syngeneic, allogeneic, and xenogeneic natural killer cells. *FASEB Journal 24*(7): 2164–2177.

Dressel, R., Schindehütte, J., Kuhlmann, T., Elsner, L., Novota, P., Baier, P.C., Schillert, A., Bickeböller, H., Herrmann, T., Trenkwalder, C., Paulus, W. & Mansouri, A. (2008). The tumorigenicity of mouse embryonic stem cells and in vitro differentiated neuronal cells is controlled by the recipients' immune response. *PLoS ONE 3*(7): e2622.

Drukker, M., Katchman, H., Katz, G., Even-Tov Friedman, S., Shezen, E., Hornstein, E., Mandelboim, O., Reisner, Y. & Benvenisty, N. (2006). Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells 24*(2): 221–229.

Drukker, M., Katz, G., Urbach, A., Schuldiner, M., Markel, G., Itskovitz-Eldor, J., Reubinoff, B., Mandelboim, O. & Benvenisty, N. (2002). Characterization of the expression of MHC proteins in human embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America 99*(15): 9864–9869.

Eapen, M., Rubinstein, P., Zhang, M.-J., Stevens, C., Kurtzberg, J., Scaradavou, A., Loberiza, F.R., Champlin, R.E., Klein, J.P., Horowitz, M.M. & Wagner, J.E. (2007). Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet 369*(9577): 1947–1954.

Eliopoulos, N. & Stagg, J. (2005). Allogeneic marrow stromal cells are immune rejected by MHC class I–and class II–mismatched recipient mice. *Gene Therapy 106*(13): 4057–4065.

Fändrich, F., Lin, X., Chai, G.X., Schulze, M., Ganten, D., Bader, M., Holle, J., Huang, D.-S., Parwaresch, R., Zavazava, N. & Binas, B. (2002). Preimplantation-stage stem cells induce long-term allogeneic graft acceptance without supplementary host conditioning. *Nature Medicine 8*(2): 171–178.

Francois, M., Romieu-Mourez, R., Stock-Martineau, S., Boivin, M., Bramson, J. & Galipeau, J. (2009). Mesenchymal stromal cells cross-present soluble exogenous antigens as part of their antigen presenting cell properties. *Blood 114*(13): 1–25.

Frenzel, L.P., Abdullah, Z., Kriegeskorte, A.K., Dieterich, R., Lange, N., Busch, D.H., Krönke, M., Utermöhlen, O., Hescheler, J. & Sarić, T. (2009). Role of natural-killer group 2 member D ligands and intercellular adhesion molecule 1 in natural killer cell-mediated lysis of murine embryonic stem cells and embryonic stem cell-derived cardiomyocytes. *Stem Cells 27*(2): 307–316.

Fujisaki, J., Wu, J., Carlson, A. & Silberstein, L. (2011). In vivo imaging of Tregs providing immune privilege to the hematopoietic stem cell niche. *Nature 474*(7350): 216–219.

Garfias, Y., Nieves-Hernandez, J., Garcia-Mejia, M., Estrada-Reyes, C. & Jimenez-Martinez, M.C. (2012). Stem cells isolated from the human stromal limbus possess immunosuppressant properties. *Molecular Vision 18*: 2087–95.

Grinnemo, K., Agneta, M.K., Skottman, M.H., Hao, X., Andersson, S.A., Stromberg, A., Lahesmaa, R., Hovatta, O., Sylven, C. & Deilgren, G. (2006). Human embryonic stem cells are immunogenic in allogeneic and xenogeneic settings. *Biomedicine 13*(5): 712–24.

Gu, Y.Z., Xue, Q., Chen, Y.J., Yu, G.H., Qing, M. De, Shen, Y., Wang, M.Y., Shi, Q. & Zhang, X.G. (2013). Different roles of PD-L1 and FasL in immunomodulation mediated by human placenta-derived mesenchymal stem cells. *Human Immunology 74*(3): 267–276.

Guha, P., Morgan, J.W., Mostoslavsky, G., Rodrigues, N.P. & Boyd, A.S. (2013). Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. *Cell Stem Cell 12*(4): 407–412.

Holan, V. & Javorkova, E. (2013). Mesenchymal stem cells, nanofiber scaffolds and ocular surface reconstruction. *Stem cell Reviews 9*(5): 609–19.

Holan, V., Pokorna, K., Prochazkova, J., Krulova, M. & Zajicova, A. (2010). Immunoregulatory properties of mouse limbal stem cells. *Journal of Immunology 184*(4): 2124–2129.

Hori, J., Ng, T.F., Shatos, M., Klassen, H., Streilein, J.W. & Young, M.J. (2003). Neural progenitor cells lack immunogenicity and resist destruction as allografts. *Stem Cells 21*(4): 405–416.

Chan, J.L., Tang, K.C., Patel, A.P., Bonilla, L.M., Pierobon, N., Ponzio, N.M. & Rameshwar, P. (2006). Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon-γ. *Blood 107*(12): 4817–4824.

Imitola, J., Comabella, M., Chandraker, A.K., Dangond, F., Sayegh, M.H., Snyder, E.Y. & Khoury, S.J. (2004). Neural stem/progenitor cells express costimulatory molecules that are differentially regulated by inflammatory and apoptotic stimuli. *The American Journal of Pathology 164*(5): 1615–1625.

Inoue, S., Popp, F.C., Koehl, G.E., Piso, P., Schlitt, H.J., Geissler, E.K. & Dahlke, M.H. (2006). Immunomodulatory effects of mesenchymal stem cells in a rat organ transplant model. *Transplantation 81*(11): 1589–1595.

Jaiswal, S., Jamieson, C.H.M., Pang, W.W., Park, C.Y., Chao, M.P., Majeti, R., Traver, D., van Rooijen, N. & Weissman, I.L. (2009). CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell 138*(2): 271–285.

Kim, E.M., Manzar, G. & Zavazava, N. (2013). Human iPS cell-derived hematopoietic progenitor cells induce T-cell anergy in in vitro-generated alloreactive CD8(+) T cells. *Blood 121*(26): 5167–5175.

Kim, S.-Y., Cho, H.-S., Yang, S.-H., Shin, J.-Y., Kim, J.-S., Lee, S.-T., Chu, K., Roh, J.-K., Kim, S.U. & Park, C.-G. (2009). Soluble mediators from human neural stem cells play a critical role in suppression of T-cell activation and proliferation. *Journal of Neuroscience Research 87*(10): 2264–2272.

Kofidis, T., DeBruin, J.L., Tanaka, M., Zwierzchoniewska, M., Weissman, I., Fedoseyeva, E., Haverich, A. & Robbins, R.C. (2005). They are not stealthy in the heart: Embryonic stem cells trigger cell infiltration, humoral and T-lymphocyte-based host immune response. *European Journal of Cardio-Thoracic Surgery 28*(3): 461–466.

Koch, C. a, Geraldes, P. & Platt, J.L. (2008). Immunosuppression by embryonic stem cells. *Stem Cells 26*(1): 89–98.

Le Blanc, K., Rasmusson, I., Sundberg, B., Götherström, C., Hassan, M., Uzunel, M. & Ringdén, O. (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet 363*(9419): 1439–1441.

Le Blanc, K., Tammik, C., Rosendahl, K., Zetterberg, E. & Ringdén, O. (2003). HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Experimental Hematology 31*(10): 890–896.

Li, L., Baroja, M.L., Majumdar, A., Chadwick, K., Rouleau, A., Gallacher, L., Ferber, I., Lebkowski, J., Martin, T., Madrenas, J. & Bhatia, M. (2004). Human embryonic stem cells possess immune-privileged properties. *Stem Cells 22*(4): 448–456.

Lu, Q., Yu, M., Shen, C., Chen, X., Feng, T., Yao, Y., Li, J., Li, H. & Tu, W. (2014). Negligible immunogenicity of induced pluripotent stem cells derived from human skin fibroblasts. *PLoS ONE 9*(12): e114949.

Lu, S.J., Li, F., Vida, L. & Honig, G.R. (2004). CD34+CD38- hematopoietic precursors derived from human embryonic stem cells exhibit an embryonic gene expression pattern. *Blood 103*(11): 4134–4141.

Ma, S., Xie, N., Li, W., Yuan, B., Shi, Y. & Wang, Y. (2014). Immunobiology of mesenchymal stem cells. *Cell Death and Differentiation 21*(2): 216–25.

Maccario, R., Podestà, M., Moretta, A., Cometa, A., Comoli, P., Montagna, D., Daudt, L., Ibatici, A., Piaggio, G., Pozzi, S., Frassoni, F. & Locatelli, F. (2005). Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. *Haematologica 90*(4): 516–525.

McDonald, J.W., Liu, X.Z., Qu, Y., Liu, S., Mickey, S.K., Turetsky, D., Gottlieb, D.I. & Choi, D.W. (1999). Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nature Medicine 5*(12): 1410–1412.

McLaren, F.H., Svendsen, C.N., Van der Meide, P. & Joly, E. (2001). Analysis of neural stem cells by flow cytometry: cellular differentiation modifies patterns of MHC expression. *Journal of Neuroimmunology 112*(1-2): 35–46.

Meisel, R., Zibert, A., Laryea, M., Göbel, U., Däubener, W. & Dilloo, D. (2004). Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood 103*(12): 4619–4621.

Morizane, A., Doi, D., Kikuchi, T., Okita, K., Hotta, A., Kawasaki, T., Hayashi, T., Onoe, H., Shiina, T., Yamanaka, S. & Takahashi, J. (2013). Direct comparison of autologous and allogeneic transplantation of IPSC-derived neural cells in the brain of a nonhuman primate. *Stem Cell Reports 1*(4): 283–292.

Nasef, A., Mathieu, N., Chapel, A., Frick, J., François, S., Mazurier, C., Boutarfa, A., Bouchet, S., Gorin, N.-C., Thierry, D. & Fouillard, L. (2007). Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. *Transplantation 84*(2): 231–237.

Park, K.-M., Cha, S.-H., Ahn, C. & Woo, H.-M. (2013). Generation of porcine induced pluripotent stem cells and evaluation of their major histocompatibility complex protein expression in vitro. *Veterinary Research Communications 37*(4): 293–301.

Polo, J., Liu, S., Figueroa, M. & Kulalert, W. (2010). Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nature 28*(8): 1–10.

Poncelet, A.J., Vercruysse, J., Saliez, A. & Gianello, P. (2007). Although pig allogeneic mesenchymal stem cells are not immunogenic in vitro, intracardiac injection elicits an immune response in vivo. *Transplantation 83*(6): 783–790.

Popp, F., Eggenhofer, E. & Renner, P. (2008). Mesenchymal stem cells can induce long term acceptance of solid organ allografts in synergy with low dose mycophenolate. *Transplant Immunology 9*(8): 1760–1772.

Ren, G., Zhang, L., Zhao, X., Xu, G., Zhang, Y., Roberts, A.I., Zhao, R.C. & Shi, Y. (2008). Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell 2*(2): 141–150.

Renner, P., Eggenhofer, E., Rosenauer, A., Popp, F.C., Steinmann, J.F., Slowik, P., Geissler, E.K., Piso, P., Schlitt, H.J. & Dahlke, M.H. (2009). Mesenchymal stem cells require a sufficient, ongoing immune response to exert their immunosuppressive function. *Transplantation Proceedings 41*(6): 2607–2611.

Rocha, V., Cornish, J., Sievers, E.L., Filipovich, A., Locatelli, F., Peters, C., Remberger, M., Michel, G., Arcese, W., Dallorso, S., Tiedemann, K., Busca, A., Chan, K.W., Kato, S., Ortega, J., Vowels, M., Zander, A., Souillet, G., Oakill, A., Woolfrey, A., Pay, A. L., Green, A., Garnier, F., Ionescu, I., Wernet, P., Sirchia, G., Rubinstein, P., Chevret, S., Gluckman, E. (2001). Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood 97*(10): 2962–2971.

Sato, K., Ozaki, K., Oh, I., Meguro, A., Hatanaka, K., Nagai, T., Muroi, K. & Ozawa, K. (2007). Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood 109*(1): 228–234.

Suárez-Álvarez, B., Rodriguez, R.M., Calvanese, V., Blanco-Gelaz, M. a., Suhr, S.T., Ortega, F., Otero, J., Cibelli, J.B., Moore, H., Fraga, M.F. & López-Larrea, C. (2010). Epigenetic mechanisms regulate MHC and antigen processing molecules in human embryonic and induced pluripotent stem cells. *PLoS ONE 5*(4): e10192.

Swijnenburg, R.J., Tanaka, M., Vogel, H., Baker, J., Kofidis, T., Gunawan, F., Lebl, D.R., Caffarelli, A.D., De Bruin, J.L., Fedoseyeva, E. V. & Robbins, R.C. (2005). Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium. *Circulation 112*(9 Suppl): 166–173.

Takizawa, H., Regoes, R.R., Boddupalli, C.S., Bonhoeffer, S. & Manz, M.G. (2011). Dynamic variation in cycling of hematopoietic stem cells in steady state and inflammation. *The Journal of Experimental Medicine 208*(2): 273–84.

Tian, L., Catt, J.W., O'Neill, C. & King, N.J. (1997). Expression of immunoglobulin superfamily cell adhesion molecules on murine embryonic stem cells. *Biology of Reproduction 57*(3): 561–568.

Tse, W.T., Pendleton, J.D., Beyer, W.M., Egalka, M.C. & Guinan, E.C. (2003). Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation 75*(3): 389–397.

Verloes, A., Van de Velde, H., LeMaoult, J., Mateizel, I., Cauffman, G., Horn, P. a, Carosella, E.D., Devroey, P., De Waele, M., Rebmann, V. & Vercammen, M. (2011). HLA-G expression in human embryonic stem cells and preimplantation embryos. *Journal of Immunology 186*(4): 2663–2671.

Waterman, R.S., Tomchuck, S.L., Henkle, S.L. & Betancourt, A.M. (2010). A new mesenchymal stem cell (MSC) paradigm: Polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PLoS ONE 5*(4): e10088.

Wu, D.C., Boyd, A.S. & Wood, K.J. (2008). Embryonic stem cells and their differentiated derivatives have a fragile immune privilege but still represent novel targets of immune attack. *Stem Cells 26*(8): 1939–1950.

Yachimovich-Cohen, N., Even-Ram, S., Shufaro, Y., Rachmilewitz, J. & Reubinoff, B. (2010). Human embryonic stem cells suppress T cell responses via arginase I-dependent mechanism. *Journal of Immunology 184*(3): 1300–1308.

Zhang, Y., Li, C.X. & Sun, L. (2011). Investigation of immunogenicity of cryopreserved limbal stem cells. *International Journal of Ophthalmology 6*(4): 590–593.

Zhao, T., Zhang, Z.-N., Rong, Z. & Xu, Y. (2011). Immunogenicity of induced pluripotent stem cells. *Nature 474*(7350): 212–215.

Zhao, Y., Wang, H. & Mazzone, T. (2006). Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics. *Experimental Cell Research 312*(13): 2454–2464.

Zheng, J., Umikawa, M., Zhang, S., Huynh, H., Silvany, R., Chen, B.P.C., Chen, L. & Zhang, C.C. (2011). Ex vivo expanded hematopoietic stem cells overcome the MHC barrier in allogeneic transplantation. *Cell Stem Cell 9*(2): 119–130.

Zheng, X.-S., Yang, X.-F., Liu, W.-G., Pan, D.-S., Hu, W.-W. & Li, G. (2007). Transplantation of neural stem cells into the traumatized brain induces lymphocyte infiltration. *Brain Injury 21*(3): 275–278.