

Review

# Immunogenicity in Stem Cell Therapy for Cardiac Regeneration

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Despite enormous advances in the treatment of cardiovascular disease (CVD), heart disease remains the leading cause of mortality and morbidity worldwide. Thus, there is a need for novel CVD therapeutics. CVD appears to be a custom-made scenario for applying stem cell therapy. Although human pluripotent stem cells can differentiate into cardiomyocytes to regenerate injured heart tissue and restore post-myocardial infarction cardiac function, several obstacles need to be overcome before cell therapy can be applied in CVD patients. One of these major hurdles is the immunological barrier. Currently, long-term immunosuppressant treatment is necessary for allogenic stem cell or organ transplantation to prevent rejection. However, the long-term use of immunosuppressants may cause serious adverse events such as nephrotoxicity, severe infections and malignancy. Thus, overcoming this immunological hurdle is crucial for the clinical application of stem cell therapy in cardiac regeneration. This review summarizes the recent advances and challenges of immunogenicity in relation to stem cell therapy.

**Key Words:** Cardiovascular diseases • Cell therapy • Major histocompatibility complex • Pluripotent stem cells • Transplant rejection

## INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death globally, accounting for approximately one third of deaths worldwide per year.<sup>1,2</sup> Myocardial infarction (MI) is one of the most serious results of CVD. MI leads to cardiomyocyte death, but the injured heart itself is unable to replace the dead tissue and has a very limited capability to regenerate new cardiomyocytes, resulting

in significant declines in contractility and cardiac output.<sup>3-5</sup> To maintain cardiac output for adequate tissue perfusion, the heart tries to make up for its lost pumping ability, but this compensation will lead to heart failure and death.<sup>6</sup> Despite enormous advances in heart failure therapies since last decade, the mortality rate of end-stage heart failure patients is still very high. Although heart transplantation is the final step to treat end-stage heart failure, only a few patients have the opportunity to get a new heart. Therefore, cell therapy seems to be a promising therapeutic option.<sup>7</sup>

The application of stem cell therapy is keenly anticipated, because stem cells can not only continually multiply but also have the potency of differentiation, so that they can turn into any cell in the body. Via endogenous or exogenous mechanisms, stem cells could repair the injured myocardium and/or restore cardiac pump function by replacing scar tissues with functionally integrated cardiomyocytes derived from stem cells.<sup>7-10</sup> According to the theory of endogenous cell therapy, endogenous myocardial precursor cells would be induced to differen-

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tiate into cardiomyocytes through mechanisms related to paracrine signaling, recombinant protein, miRNA, and exosomes, although the therapeutic effect is not yet consistent.<sup>7,9,10</sup> Exogenous methods try to use cardiomyocytes derived from pluripotent stem cells (PSCs), i.e. embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), to transplant and replace the damaged myocardium. Many animal studies have demonstrated the promising therapeutic efficacy of post-MI cell transplantation to regenerate the infarcted myocardium, to reduce the fibrotic area, and to improve left ventricular function.<sup>11-15</sup>

In spite of the promising therapeutic efficacy of PSC-derived cardiomyocyte (PSC-CM) transplantation, many obstacles need to be overcome. One of these major barriers to the successful clinical use of PSC-CM transplantation is immune rejection.<sup>9,14,15</sup> Allogeneic transplantation has a high chance of major histocompatibility complex (MHC) antigen mispairing between donor and recipient, leading to a series of immune responses and eventually graft rejection. Hence, strong immunosuppressive agents are necessary to prevent post-transplant rejection, but the long-term administration of strong immunosuppressants is often accompanied with dangerous side effects, such as fatal infections.<sup>15</sup> On the other hand, if the immunosuppressant dose is not enough to prevent rejection, then the transplanted graft will be lost, leading to organ failure.<sup>15</sup>

This review discusses the recent evidence of immune limitations and the progress of PSC-CM transplantation.

## PROGRESS AND HURDLES OF STEM CELLS IN CARDIAC REGENERATION

To date, only ESCs and iPSCs have been proven to differentiate into cardiomyocytes. ESCs are derived from the cell mass taken from the blastocyst stage of the embryo. However, producing ESCs requires sacrificing an entire fertilized egg, so that using human ESCs for research or therapeutic purposes has been constrained by complex social and ethical considerations. In 2007, Yamanaka's group reprogrammed somatic cells into iPSCs by transfecting four transcription factors (Sox2, Oct3/4, Klf4, and c-Myc). These iPSCs had the ability to differentiate into three germ layers and each type of organ tis-

sue.<sup>16</sup> This major breakthrough led to a rapid progress in research on regenerative medicine.

Wingless-type signaling can be manipulated to induce cardiac differentiation of PSCs for the large-scale production of contracting cardiomyocytes.<sup>11,12,15,17</sup> These human PSC-CMs have been shown to sufficiently engraft into damaged hearts and result in favorable effects on cardiac function.<sup>12-15,18</sup> Because primary cardiomyocytes will lose their abilities of contraction and proliferation, PSC-CMs have been considered to be a favorable alternative for cell therapy.

Although xenografts of human iPSC-CMs have shown good therapeutic effects in post-MI animal models, prior to the clinical application of human PSC-CM transplantation, significant obstacles including post-transplant tumor formation, arrhythmias and immune rejection need to be overcome.<sup>19</sup> First, the most efficient method to transfect Yamanaka factors is through viral vectors, which carries the risk of deoxyribonucleic acid (DNA) mutation. Although abnormal karyotypes or mutant cells must not be detected prior to transplantation, the possibility of post-transplant mutations cannot be completely ruled out.<sup>20</sup> Additionally, PSC transplantation has been shown to cause teratomas or teratocarcinoma formation *in vivo*. Second, several non-human primate studies have identified sustained and/or non-sustained ventricular tachycardia episodes after the intramyocardial transplantation of PSC-CM grafts, although mechano-electrical coupling was identified between the grafts and the host tissues.<sup>14,15,21</sup> Third, immune rejection is the most serious problem after allogeneic organ or tissue transplantation. Therefore, immunosuppressive drugs are mandatory to avoid graft rejection. However, there are significant drawbacks to the long-term use of immunosuppressive drugs, such as life-threatening infections, malignancy and renal failure.<sup>19,22</sup>

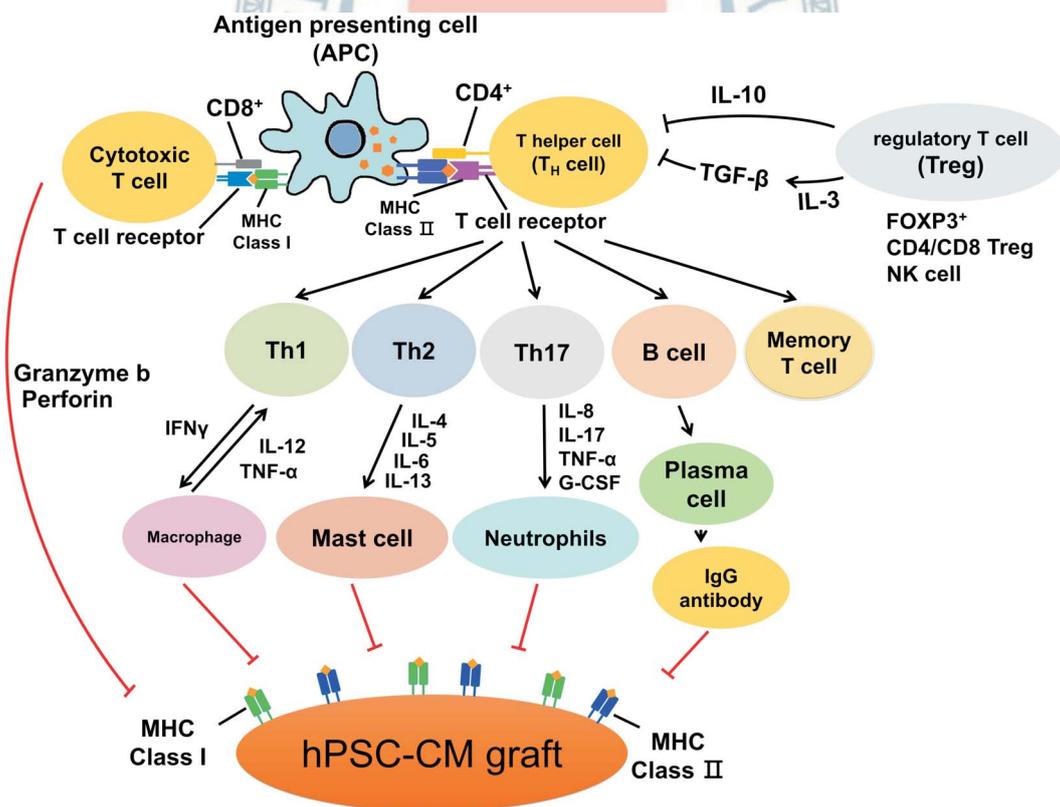
## IMMUNE RESPONSE AFTER PSC-CM TRANSPLANTATION

Immune rejection is a tandem reaction caused by immune cells that attack the graft through cytokine release, inflammation, cytotoxic mechanisms, and phagocytosis. The process of immune rejection can be divided into two stages. In the sensitization stage, the recipient's

lymphocytes identify antigens on the graft and become activated and proliferate. The second stage, called the effector stage, entails the destruction of the graft. The rejection progress and level depend on the tissues and organs involved.<sup>23</sup> Throughout the reaction, T cells are the main cells that initiate rejection, and they also act as the adaptors of secondary lymphocytes. Moreover, rejection can be divided into acute rejection and chronic rejection. Acute rejection usually begins within the first few weeks after transplantation.<sup>24</sup> This type of rejection is typically caused by syngeneic grafts and occurs approximately 10 days after transplantation. Pathological sections show significant lymphocyte infiltration and the tissue is destroyed by macrophages. In comparison, patients with chronic rejection have a reduced acute response through the use of immunosuppressants, but

the rejection occurs after a long period of time or even many years later.

Human MHC, known as human leukocyte antigen, plays a major role in the immune rejection response after tissue or organ transplantation (Figure 1). Usually, the severity of the immune rejection response depends on the percentage of MHC mis-match.<sup>25</sup> Human MHC proteins can be divided into two major categories. MHC class I molecule is located on all nucleated cells in the body, while MHC class II molecule is distributed only on antigen-presenting cells (APCs), such as macrophages, B cells, and dendritic cells. Furthermore, MHC class I and II molecules mediate CD8<sup>+</sup> and CD4<sup>+</sup> T cell activation, respectively.<sup>23</sup> In humans, T cell-mediated hypersensitivity and cytotoxicity play important roles in graft rejection. The donor dendritic cells, acting as APCs, reveal the



**Figure 1.** The mechanism of immune rejection caused by major histocompatibility complex (MHC) mismatch. The immune response post pluripotent stem cell-derived cardiomyocyte (PSC-CM) transplanted is initiated by the mismatch of MHC class I and class II between the graft and the host T cells. If the graft is recognized as a foreign object, these T cells will initiate a series of reactions, including the classical CD4 pathway and secondary reaction, such as helper T cell (Th)1, Th2, Th17, cytotoxic T cells and regulate T cells-mediated cytokines. During the immune response, Treg also activate to prevent excessive activation immune cells to attack host cells. APC, antigen-presenting cell; CD4<sup>+</sup>, helper CD4<sup>+</sup> T cells; CD8<sup>+</sup>, cytotoxic CD8<sup>+</sup> T cells; FOXP3, forkhead box P3; G-CSF, granulocyte colony stimulating factor; hPSC-CM, human pluripotent stem cells derived cardiomyocyte; IFN-γ, interferon gamma; IL, interleukin; MHC, major histocompatibility complex; hPSC-CM, human pluripotent stem cells derived cardiomyocyte; IFN-γ, interferon gamma; IL, interleukin; MHC, major histocompatibility complex; NK cell, natural killer T cell; TGF-β, transforming growth factor beta; Th, helper T cell; TNF-α, tumor necrosis factor-α; Treg, regulatory T cells.

MHC peptide on the cell surface. APCs present MHC-II antigen fragments on their surface, which are then recognized by helper T cell (Th) receptors. If an incorrect antigen pairing is confirmed, the Th cells will proliferate and differentiate into functional Th cells, i.e. effector Th cells and memory T cells. In contrast to CD4<sup>+</sup> T cells, the process of CD8<sup>+</sup> T cell activation is simpler. Pre-CD8<sup>+</sup> T cells express an MHC-I receptor that binds to the MHC-I antigen, thereby causing differentiation and producing mature cytotoxic T lymphocytes (CTLs). These activated CTLs dissolve the transplanted tissue by inducing target cell apoptosis through the release of granzyme B and perforin.<sup>26,27</sup>

### CD4<sup>+</sup> T CELL-MEDIATED IMMUNE RESPONSE

In addition to direct immune responses, CD4<sup>+</sup> T cells also mediate multiple reactions, such as the release of interleukin (IL)-6, which is expressed by CD4<sup>+</sup> T cells during the acute inflammatory phase after transplantation. IL-6 is secreted and then acts to increase the function of CD4<sup>+</sup> T cells. IL-6 neutralization has been shown to alter Th1 responses and reduce serum antibodies, leading to a delay in the onset of acute rejection and considerably prolonged graft survival duration.<sup>28</sup>

Furthermore, IL-17, secreted by Th17 cells, is also indirectly induced by activated CD4<sup>+</sup> T cells and is abundantly expressed in the acute inflammatory phase after transplantation. When a foreign antigen is present, IL-6, IL-21, and IL-23 induce Th17 cell differentiation. IL-17A and IL-17F will then be released by target innate immune cells and epithelial cells in the early stages of rejection when Th17 cells have been activated. Additionally, G-CSF and IL-8 can be secreted and will recruit neutrophils to produce acute inflammation.<sup>29</sup>

### THE ROLE OF REGULATORY T CELLS (Tregs) IN IMMUNE RESPONSE

In addition to the classic rejection pathways, Tregs are a group of lymphocytes with immunoregulatory function which can help maintain the body's immune tolerance to prevent the excessive activation of immune responses. Regulatory T cells can be divided into naturally

occurring and naturally regulated T cells and induced adaptive regulatory T cells. Moreover, Tregs can be divided into central tolerance and peripheral tolerance. The former are derived from T cells during thymus gland development and are produced by encountering specific antigens. The peripheral system is self-differentiated by mature naive CD4<sup>+</sup> T cells, and this is the major functional reaction produced after organ transplantation. These two systems are not completely independent, and they regulate each other and eventually reduce the activity of Th1, Th2, and B lymphocytes to reduce the inflammatory response.<sup>30</sup> In summary, Tregs act as a regulator in allogeneic transplantation, which is promising for the prevention of acute and chronic rejection.<sup>31,32</sup>

### STEM CELL THERAPY WITH POTENTIAL IMMUNE PRIVILEGE

#### Mesenchymal stromal cells (MSCs)

An increasing number of studies have explored the clinical application of MSCs. These cells exist in several tissues and organs and have multipotent differentiation capacity *in vitro*. Although MSCs may arise from various sources and express special surface antigens, importantly, most MSCs do not express human leukocyte antigen (HLA) class II antigen, resulting in CD4<sup>+</sup> T-cell dysfunction and the lack of Th cell-dependent antibody production. Therefore, they may have the unique property of immunomodulation.<sup>33,34</sup>

Nonetheless, there are several limitations with regards to the clinical application of MSCs.<sup>35</sup> These cells cannot efficiently differentiate into real cardiomyocytes with spontaneous contraction, and MSC-derived cells are unable to synchronize with the host tissue. Furthermore, transplanted MSCs have a lower immune response, but MSC transplantation does not constantly improve cardiac function.<sup>35,36</sup>

A novel dual stem cell therapy approach was applied for post-MI cardiac regeneration.<sup>37</sup> In this approach, intramyocardially transplanted PSC-CMs and epicardially implanted MSC patches led to enhancement of vessel formation, improving the retention and maturation of PSC-CMs and a considerable improvement in post-MI cardiac function. In addition, the MSC patches provided not only a microenvironment to enhance vascular re-

generation and increase PSC-CM retention, but also pleiotropic effects, including anti-fibrosis and anti-inflammation.<sup>37</sup> Notwithstanding the promising results of this dual stem cell therapy, it should be noted that this approach was examined in a rat MI model with permanent ischemia, and the outcomes would be different in a clinically relevant MI model of myocardial ischemia-reperfusion. Therefore, further studies using large animals with myocardial ischemia-reperfusion models are needed.

### Autologous iPSCs

Theoretically, autologous iPSCs should be able to avoid immune rejection. However, genetic and epigenetic variations may also occur during reprogramming so that there is still a risk of immune rejection of autologous iPSCs transplantation.<sup>38,39</sup> Moreover, an autologous approach requires the collection of patient cells, reprogramming, selecting pluripotent cells, expansion and generation of cardiomyocytes at large scale, which would take at least 6 months to generate under good manufacturing practice conditions. Therefore, because of the high cost and long duration to generate autologous iPSCs, autologous iPSC-CM therapy for MI may not be feasible in current clinical practice.

### Potential universal stem cells without MHC I or II expression

Because incompatible MHC pairing is the major cause of immune rejection, knocking out MHC genes on grafts might be an effective way to avoid rejection. The development of gene editing technology, especially the clustered regularly interspaced short palindromic repeats/associated proteins 9 system (CRISPR-Cas9 system), has improved the accuracy and efficiency of gene knockout. Knocking out  $\beta 2$  microglobulin and the class II MHC transactivator gene of iPSCs has been shown to fail to activate T cell-mediated immune responses, but did not change the iPSCs' pluripotency.<sup>40</sup> Furthermore, no significant difference in cardiac differentiation and electromechanical properties has been reported between ESCs and iPSCs without MHC I/II expression.<sup>11,40</sup> After coculturing with peripheral blood mononuclear cells, these MHC-knockout universal stem cells only induced little activity in human immune cells.<sup>40</sup>

Nevertheless, MHC I-negative cells would be lysed by natural killer cells (NKC).<sup>41</sup> Furthermore, Nakamura

et al.<sup>42</sup> demonstrated significant NKC infiltration into engrafted tissues after intramyocardial transplantation of iPSC-CMs without the expression of MHC I molecules. According to the immune reaction mentioned above, the lack of MHC II expression may prevent the initiation of immune rejection.

Additionally, it has been demonstrated that the post-MI acute inflammatory response of hosts could be key to the therapeutic benefit of adult stem cell therapy in injured hearts.<sup>43</sup> The activation of immune response after allogeneic adult stem cell transplantation has been shown to be harmful to the graft and host. Nevertheless, Vagnozzi et al. showed that the acute post cell-transplanted immune response leading to the activation of CCR2<sup>+</sup>CX3CR1<sup>+</sup> macrophages would change the activity of fibroblasts and reduce myocardial fibrosis in the border zone of myocardial ischemia, and also increase the mechanical properties of the infarcted region.<sup>43</sup> Moreover, the advantage of post-MI stem cell therapy could be due to the wound healing response, which would rejuvenate the infarcted hearts and improve post-MI cardiac function.

### CONCLUSION

Overcoming the immunological hurdle of transplanted PSC-CMs is crucial for the clinical application of cell therapy in cardiac regeneration. Owing to the many obstacles mentioned above, a simple, stable, low-immunity rejection alternative is needed. This alternative approach should avoid excessive modifications during commercialization — such as not using viral vectors or gene editing — to reduce the likelihood of clinically unanticipated risks.

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### CONFLICTS OF INTEREST

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