REVIEW

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Immune responses to bioengineered organs

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Purpose of review
Organ donation in the United States registered 9079 deceased organ donors in 2015. This high percentage of donations allowed organ transplantation in 29 851 recipients. Despite increasing numbers of transplants performed in comparison with previous years, the numbers of patients that are in need for a transplant increase every year at a higher rate. This reveals that the discrepancy between the demand and availability of organs remains fundamental problem in organ transplantation.

Recent findings
Development of bioengineered organs represents a promising approach to increase the pool of organs for transplantation. The technology involves obtaining complex three-dimensional scaffolds that support cellular activity and functional remodeling though tissue recellularization protocols using progenitor cells. This innovative approach integrates cross-thematic approaches from specific areas of transplant immunology, tissue engineering and stem cell biology, to potentially manufacture an unlimited source of donor organs for transplantation.

Summary
Although bioengineered organs are thought to escape immune recognition, the potential immune reactivity toward each of its components has not been studied in detail. Here, we summarize the host immune response toward different progenitor cells and discuss the potential implications of using nonself biological scaffolds to develop bioengineered organs.

Keywords
allogeneicity, bioengineered organs, extracellular matrix, stem cells

INTRODUCTION

Organ donation in the United States registered 9079 deceased organ donors in 2015 according to the 'Newsletter Transplant', (September 2016, Vol. 21). This high percentage of donations allowed organ transplantation in 29 851 recipients: 24 225 kidney, 7127 liver, 2819 heart, 2072 lung, 947 pancreas and 141 small bowel. Despite increasing numbers of transplants performed in comparison with previous years (2014 = 28 523 transplant recipients), the number of patients that are in need for a transplant increases every year at a higher rate, and more than 11 900 people in the United States are currently on the waiting list for a life saving organ (https://www.unos.org/). As a result, about 13 000 people in the United States alone die or become sick every year while waiting for a transplantable organ (https://optn.transplant.hrsa.gov/). In addition, 22 people die every day on average from the lack of available organs for transplant (https://www.donatelife.net/), which reveals a fundamental problem in organ transplantation: discrepancy between the demand and availability of organs.

This situation has been the major driving force behind the rise of organ engineering approach to create transplantable grafts that may potentially be used as alternatives to donor organs. In organ engineering, human cadaveric or animal organs are

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Curr Opin Organ Transplant 2017, 22:79–85

DOI:10.1097/MOT.0000000000000378

KEY POINTS

- Bioengineered organs represent a potential source of unlimited donor grafts for transplantation.
- The immune response to bioengineered organs remains largely unknown.
- Nonself ECM and stem cells used to build bioengineered organs trigger immune responses.
- Common immunosuppressive therapy may not control the immunogenicity against bioengineered organs.

perfused with a detergent solution to remove the cells leaving behind the extracellular matrix (ECM) scaffold that still maintains the microarchitecture and the composition of the native organ. The resulting structure can be later repopulated with healthy cells – adult, progenitor or pluripotent stem cell derived – to engineer transplantable and functional organ substitutes [1]. This strategy has been shown to be feasible for heart [2▪▪], liver [3], lung [4,5] and kidney [6] using animal or human cadaveric organs in a number of studies since 2008. Although it is theoretically possible to create nonimmunogenic organs by using patient-derived cells for repopulating scaffolds, the specific in-vivo immune reactivity to different subsets of stem cells and to the different components of the ECM scaffolds is not fully understood.

Here, we review the current knowledge on immune reactivity of stem cells that are potentially used to engineer transplantable organ grafts and explore potential therapies that may be employed to minimize the immune responses to bioengineered organs.

IMMUNE RESPONSE TO STEM CELLS

Stem cells have the unique capacity to self-renew and differentiate into specialized cell types, therefore providing a mean to restore tissues and functions altered by diseases and/or senescence. They are particularly suited for regenerative medicine. Among them, there are embryonic stem cells (ESCs); various primary cell types including endothelial, mesenchymal, cardiac progenitor and stem cells collectively termed adult stem cells; and induced pluripotent stem cells (iPSCs). Historically, there has been a relative lack of concern for the immune reactivity of stem cells due to the almost dogmatic concept that ESCs are immune privileged. Recently, however, stem cells immunogenicity has become a major concern, as the immune privileged status of ESCs has been questioned [7]. Although stem cells may have a demonstrable low immunogenic profile in their undifferentiated state, their progeny express human leukocyte antigen (HLA) molecules at their surface, which makes them immunogenic. Even self-derived autologous iPSCs are exposed to genetic instability and epigenetic reprograming which may alter their immune neutrality [8]. Given that obtaining autologous iPSC derived from often elderly diseased patients represents a difficult task, adult stem cells of nonself allogeneic origin have become a more realistic and pragmatic therapeutic choice [9]. The translational development of stem cell therapies requires taking into account the immune status of the cells as well as of the patient, and the immune potential of the therapeutic product in a manner similar to organ transplantation. An immunologically educated choice of the therapeutic cells as well as a pretreatment and posttreatment immune monitoring plan will need to be implemented to ensure the optimal clinical success.

HUMAN EMBRYONIC STEM CELLS

The proposed immune privilege status of human ESCs (hESCs) originates from the observation that undifferentiated hESCs do not induce allogeneic T-cell proliferation in in-vitro MLR assays [10]. However, murine ESCs (mESCs) transplanted in an allogeneic recipient are rejected, and repeated injections of allogeneic mESCs are rejected faster likely through immune memory mechanisms [11]. The mechanisms of rejection are still unclear because, despite major histocompatibility complex (MHC) class I expression of the inner cell mass of blastocyst-stage embryo was confirmed by immunohistochemistry, mESCs were not sensitive to natural killer (NK) cell killing [12]. Although the in-vitro and in-vivo ESC derivatives express low levels of MHC class I molecules, IFN treatment results in MHC class I increase on both ESCs and their derivatives, which may also increase their immunogenicity [12]. These initial experiments anticipated various strategies to overcome the allogeneicity of ESCs, which included the development of a universal cell model devoid of MHC class I proteins. Several groups have proposed to knockout β2m and observed that the loss of MHC class I on those hESCs render them resistant to $CD8^+$ T-cell rejection [13]. This strategy was extended to the inducible expression of MHC class II by deleting its master regulator the CIITA (class II trans-activator) [14▪]. Although the combined knockout of β2m and CIITA are effective from an immune standpoint, it is unlikely that these engineered cells and their derivatives will be used therapeutically as any cell lacking MHC will not be eliminated when infected by a virus or undergo an oncogenic event. Other approaches to obtain immune insensitive ESCs that

include the creation of nuclear transfer human hESC cell lines along with common immunosuppressive regimen or costimulatory blockade for the induction of tolerance has been proposed [15–17].

INDUCED PLURIPOTENT STEM CELLS

Although autologous in origin, the iPSCs display a yet unpredictable array of genetic and epigenetic modifications leading to phenotypic abnormalities likely to be recognized as such by the immune system of the recipient. Although the main concern is tumorigenicity, the intricate immunogenicity has to be taken in account for their clinical development. The expression of MHC molecules and other molecules, such as Tapasin or transporter associated with antigen processing 1, which are required for proper expression of MHC class I, are low in iPSCs as a result of the reprograming procedure. An initial report stressed the capacity of iPSCs to elicit specific syngeneic T-cell responses in C57BL/6 mice [8]. Although further studies claimed that tissue (skin) derived from iPSCs may have low immunogenic profile, the expression and stability of low expression profile of MHC molecules cannot be guaranteed particularly within inflammatory and/or hypoxic environment. In contrast to autologous iPSCs, allogeneic iPSCs are likely to drive an allogeneic immune response, as even low levels of MHC expression are sufficient to be recognized by the host immune system by the direct or indirect allorecognition pathways. The immunogenic potential of allogeneic iPSCs is likely to vary according to the reprograming protocol, where some procedures may minimize the genetic and/or the epigenetic variability and instability. The search for a transcriptional signature associated with lower immunogenicity would be indeed of value in selecting the optimal iPSCs for therapy. Therefore, the allogeneicity of iPSCs should be avoided or controlled to achieve an effective and nondetrimental use of iPSCs. The potential immunological response of the host cells may be lowered by common immunosuppressants that regulate effector mechanisms during T-cell responses. Thus, the capacity for allogenicity is intrinsic to any cell expressing the MHC molecules, whereas the consequences at the effector T-cell level may be modulated or suppressed. Furthermore the use of autologous iPSCs being a long and expensive process, the use of iPSCs from HLA homozygous donors appears to be a more realistic development in human regenerative medicine.

CARDIAC STEM CELLS

Transplantation of cardiac stem/progenitor cells represents a promising source of cells for organ bioengineering. The proof-of-concept obtained in animal models showing attenuated left ventricular remodeling and improved ventricular function after injection of various types of cardiac stem cells (CSCs) led to test different cell populations including autologous adult CSCs or cardiosphere-derived cells in meaningful clinical trials. However, the biological limitation of the autologous setting and the logistical constraints call upon the use of allogeneic products. The mechanism(s) regulating the behavior of allogeneic CSCs has been only recently investigated with the aim of evaluating the risk and validating their clinical potential. We investigated the immune status of allogeneic hCSCs in the prospect of using them clinically, as the allogeneic option was favored for logistic reasons (principally off-the-shelf availability and quality control). hCSCs express low levels of MHC class I molecules, which can be upregulated under inflammatory and hypoxic conditions mimicking the local environment of ischemia-reperfusion following organ transplantation. Under these conditions, we observed that hCSCs express significant levels of the costimulatory molecule programmed death ligand 1 (PDL1) and IL10, which results in immune regulation [18]. This tolerogenic phenomenon was shown to be the result of a cell contact-dependent expansion and activation of regulatory T cells through the PD1–PDL1 pathway, which was amplified under inflammatory conditions. In addition, hypoxic hCSCs are also able to downregulate NK cell–mediated cytotoxicity and to polarize NK cells toward secretion of anti-inflammatory cytokines IL10 and IL13 rather than inflammatory IFNγ and TNFα [19]. We therefore proposed the benefits of using allogeneic cells maintained through hypoxic conditions with the potential paracrine effect on endogenous cardiogenesis [9]. This promoted the entry of allogeneic hCSC to an ongoing clinical trial.

Cardiosphere-derived cells constitutively express MHC classes I and II molecules in an IFNγ-inducible manner but do not express CD80/86 costimulatory molecules. This immune profile would prevent NK cell–mediated cytotoxicity and favor anergic T-cell responses. A recent report showed, in a rat model of acute myocardial infarction, that two consecutive injections of allogeneic cardiosphere-derived cells would not elicit a detectable immune response and produce a cardiac benefit without sign of rejection or humoral immune sensitization [20▪]. The proliferative response and cytokine release were identical in autologous and allogeneic settings, therefore providing experimental argument that there may be no need of immunosuppressive therapy. These results are in line with the concept of allogeneic-driven benefit and the mechanism described above for

adult hCSC. A cautionary note, however, is that this favorable immune status will need to be maintained for a sufficient length of time to allow the beneficial cardiac effect and should not be compromised by recurrent infectious or inflammatory conditions.

ADULT STEM CELLS (TISSUE STEM CELLS)

Adult or, as often called, tissue stem cells have been identified throughout the years in several organs and tissues, including liver [21–23], lungs [24,25], bone marrow [26–31], blood vessels [32–34], skeletal muscle [35–38], intestine and colon [39], skin [40–42] and heart [43,44], just to mention a few. In tissues with slow cell turnover (like the heart, brain or liver), they are found quiescent in specific areas of each tissue designated as stem cell niche and are activated after injury. In organs with faster cellular turnover (like the skin or the intestine), they are constantly dividing and generating more differentiated progeny [45] to quickly replace short living mature cells.

In the past 3 decades, tissue engineers and stem cell biologists have been exploring the most adequate ways to harness the inherent power of these cells to create new tissues and treat human diseases. However, our knowledge of their immunogenicity upon transplantation is still quite incomplete. Particularly, in the field of bioengineered organs, our basic science and clinical experience is still quite limited. Nonetheless, some experimental work on their behavior *in vitro* and *in vivo* upon cellular transplantation is providing new insights into the specific mechanisms that are regulating host immune's response. Adult and fetal liver stem cells are a good example of this.

The required procedures to isolate liver stem cells from fetal and postnatal donors and their inherent biology are generally known today [21,46▪▪]. In addition, even more primitive stem cells have been identified throughout the human biliary tree stem cells (hBTSCs) with the potential to generate hepatocytes, cholangiocytes, pancreatic islets and intestinal cells [22,47]. Independently of their donor origin, they seem to have low immunogenicity, and it is feasible to transplant them without the use of immunosuppression into human patients [48,49▪]. The lack of signs of rejection and/or allergy without any immunosuppressive treatment, seems to correlate with marginal or absence of expression of HLA classes I and II antigens both in hepatic and biliary tree stem cells from fetal liver [50–52].

Also, Riccio *et al.* [50] data suggest that hBTSCs could modulate the T-cell response through the secretion of FasL, which impacts the lymphocyte Fas/FasL pathway by producing 'early' apoptosis in $CD4^+$ and $CD8^+$ T cells. In addition, Maraldi *et al.* [52] proposed that hepatocyte growth factor secreted by hBTSC also exerts a cytoprotective role by stimulating apoptosis in these same human immune cells. Finally, Bruno *et al.* [51] have shown that hBTSC can inhibit T-cell proliferation by releasing prostaglandin E2, impair dendritic cell differentiation from monocytes and inhibit NK-cell degranulation. Hence, all these immunomodulatory processes hint at some of the core mechanisms how these human fetal liver and hBTSC (despite their apparent different origin) control immune surveillance toward themselves. Though, the critical question that remains is what exactly happens *in vivo* after cell transplantation that maintains these cells viable in the target organ for months [53]. This question, combined with an expected slight increase in the levels of expression of HLA type I during the stem cell differentiation process suggest a more complex mechanism of immunomodulation, which is not completely understood and whose maintenance is still quite unknown in differentiated stem cells seeded in bioengineered organs. Hence, without further supporting research, it is quite conceivable that unlike primitive stem cells, long-term protection from immune system–mediated killing could be an overconfident outlook in the future development of bioengineered organs-based therapeutics.

MESENCHYMAL STEM CELLS

Despite the current difficulties in predicting the immune response to bioengineered organs, the increasing clinical use of allogeneic MSCs with their immunomodulatory properties is probably a game changer. Their use in bioengineered organs has the advantage of adding an important 'plastic' stromal cell population with the potential to generate important cell lineages and shape the tissue (smooth muscle cells, 'fibroblasts' and pericytes). In addition, their true potential also resides in their ability to induce immune tolerance to the bioengineered tissues/organs [54]. This review is not specifically addressing their nomenclature or functional characteristics, their origin or prolific heterogeneity. Nevertheless, in spite of the common properties of MSCs listed by the International Society for Cellular Therapy [55], important differences and molecular signatures have been observed between MSCs that were derived from different tissues [56,57]. This helps us to postulate the use of tissue-specific MSCs in organ bioengineering to harness MSCs's unique tissue identity for effective organ reconstruction. Yet, when considering trophic and immunomodulation alone, it seems far easier and reasonable to rely on 'universal donor' allogeneic MSCs to gain these effects [54]. Although it is unclear at this point

whether allogeneic MSCs in the bioengineered organs will stimulate immune tolerance or will persist following transplantation [58,59].

CONCLUSION

The evolving paradigm of tissue/organ bioengineering presents new challenges to bioengineers, when addressing the large numbers of cells and resources needed to make bioengineered solid organs a clinical reality. The commercial implementation of scale-out approaches to produce autologous therapies has been difficult to implement due to the regulatory landscape, inherent costs and complexity [60]. Hence, some of the present-day interrogations and debate are focused on the adoption of autologous versus allogeneic cells in the bioengineering of tissues and organs, with the anticipated consequences in the patient's life due to the required immunosuppressive therapies when the 'allogeneic option' is favored. However, considering the current reality and despite some recent progress [43,44,46▪▪], it is virtually impossible to generate the necessary billions of cells to bioengineer a human liver [61] or a heart [2▪▪] from isolated adult stem cell. Consequently, hESC and hiPSC represent a more realistic alternative available to fulfill this endeavor. This represents a potential problem, as the innate and the cellular immune response to stem cells are not fully understood. In addition, the humoral immune response following injection of allogeneic stem cells needs to be taken into account [62]. The presence of anti-HLA antibodies after hESc and hiPSC therapy and preformed antibodies prior to injection might compromise and limit the survival and the efficacy of the bioengineered organ. Hence, unknown consequences of the potential immunogenicity of these bioengineered organs created with allogeneic hESC and hiPSC-derived cells is gaining relevance and shifts the focus of the debate from the use of autologous cells to the inherent control of their immunogenicity.

The immune reactivity to decellularized scaffolds that are composed of ECM, also need to be taken into account, especially when derived from allogeneic or xenogeneic tissues. The immune reactivity of ECM has been studied in the past to conclude that ECM of xenogeneic origin is immunogenic. Allaire *et al.* [63] demonstrated in 1997 that the immunogenicity of arterial xenografts is supported by the ECM, which suggests that interspecies, but not an intraspecies graft antigenicity is induced by ECM transplant [64]. A recent review from Keane and Badylak [65▪▪] summarizes the current immunological implications associated with the use of biological scaffolds. Indeed, several reports describe that each of the ECM components activate the innate immune response: proteoglycans (heparan sulfate, chondroitin sulfate and keratan sulfate) [66]; nonproteoglycan polysaccharide (hyaluronic acid) [67▪]; fibers (collagen and elastin) [68,69] and others (fibronectin and laminin) [70]. The immune reactivity against the ECM is of special interest in the context of chronic rejection, as this process is not prevented by current immunosuppressive therapy and involves multiple immunological processes in which graft ECM is slowly destroyed, whereas graft blood vessels are obstructed by the deposition of collagen [71].

Understanding how decellularized–recellularized organs maintain their function while inhibiting undesirable immune responses *in vivo* has critical implications to successfully develop this innovative technology. It remains unknown if common immune suppressive therapy, such as rapamycin or cyclosporine, is sufficient to overcome the allo/xenogeneic barrier of the transplanted bioengineered organs *in vivo*. Regardless, one should not lose perspective that sometime in a not-so-distant future, bioengineered organs will increase the pool of available organs for transplantation, but at the cost of immunosuppression until patient's specific hiPSC are easy and inexpensive to generate and produce. Therefore, immune-monitoring strategies for bioengineered organs will hopefully need to be developed in the near future.

Acknowledgements

We would like to thank Patricia Conde for her assistance with the study.

Financial support and sponsorship

The work was supported by the National Institutes of Health (R00DK088962) to B.E.U., by H2020-MSCA-IF-2014-660554 and ISCIII PI15/00563 to P.M.B., and SAF SAF2013-48834-R/SAF2016-80031-R to J.O.

Conflicts of interest

B.E.U. has a financial interest in Organ Solutions, LLC, which is reviewed and arranged by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies. The remaining authors have no conflicts of interest.

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