Review Article

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Allogenicity & immunogenicity in regenerative stem cell therapy

Dominique Charron

Laboratoire "Jean Dausset" - Laboratoire d'Immunologie et d'Histocompatibilité, Hôpital Saint Louis, CIB-HOG, AP-HP & INSERM UMRS 940, Institut Universitaire d'Hématologie, Université Paris-Diderot ,1, Avenue Claude Vellefaux, 75010, Paris, France

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The development of regenerative medicine relies in part on the capacity of stem cells to differentiate into specialized cell types and reconstitute tissues and organs. The origin of the stem cells matters. While autologous cells were initially the preferred ones the need for "off the shelf" cells is becoming prevalent. These cells will be immediately available and they originate from young non diseased individuals. However their allogenicity can be viewed as a limitation to their use. Recent works including our own show that allogenicity of stem cell can be viewed as on one hand detrimental leading to their elimination and on the other hand beneficial through a paracrine effect that can induce a local tissue regenerative effect from endogenous stem cells. Also their immune modulatory capacity can be harnessed to favor regeneration. Therefore the immune phenotype of stem cells is an important criteria to be considered before their clinical use. Immuno monitoring of the consequences of their *in vivo* injection needs to be taken into account. Transplantation immunology knowledge will be instrumental to enable the development of safe personalized regenerative stem cell therapy.

Key words Allogenicity - embryonic stem cells - HLA - immunogenicity - major histocompatibility complex - SC therapeutics

Introduction

Stem cells (SCs) have the unique capacity to differentiate into one or more specialized cell types. They have the potential to regenerate human organs altered by disease or ageing and repair injured tissues. Stem cell based regenerative therapies have raised hopes for novel therapeutic approaches[1]. This has been particularly prominent in the field of incurable heart failure, and various stem or progenitor populations have been proposed to achieve cardiac repair and regeneration[2]. SCs for regenerative medicine include embryonic stem cells (ESC)[3], induced pluripotent stem cells (iPSC) established through reprogramming of somatic cells, and various primary cell types including endothelial progenitor cells (EPC), mesenchymal stem cells (MCS), cardiac derived progenitor cells (CDP), and cardiac stem cells (CSC) collectively termed adult stem cells (adult SCs)[4]. While autologous derived cells have been initially considered the preferred ones, their limitation in term of immediate availability has questioned the feasibility of their translational use in

the clinic. Also their derivation from often elderly, diseased patients with co-morbidities has raised concerns about their suitability. In contrast, embryonic stem cells or adult stem cells derived from healthy donors have the advantage to be immediately available as an 'off the shelf' therapeutical product. Interestingly, stem cells from various origins have in addition to their regenerative power the capacity to induce endogenous *in situ* regeneration. Therefore, a paracrine effect is now to be considered as an important therapeutic element in addition to the regenerative one. The combined regenerative and paracrine effects should be investigated as intrinsic characteristics of any SC to be translated into valid therapy. There has been initially a lack of interest for potential immunological conflicts between transplanted ESC-derived tissues and host. The concept that ESCs may have an immune privilege status has gained support from trima mouse model of ESC transplantation where human embryonic stem cells were administered under the kidney capsule of recipients reconstituted with human peripheral blood leucocytes. However, it is clear that immunological rejection of transplanted ESC-derived tissues occurs frequently and that early prediction of lack of immunogenicity may be ultimately incorrect[5-7]. The models of ESC transplantation using murine ESCs showed that administration of these cells into the myocardium of allogeneic animals resulted in robust inflammatory responses and cellular infiltration by both innate and adaptive components of the immune system[8]. Today, most evidences suggest that the immunological barriers of ESC-derived cells transplantation are the same as those encountered and continue to confound solid-organ and bone marrow transplantations[9,10]. While allogenic stem cells logically qualify to induce a host immune response, there is recent evidence that autologous derived stems cells, particularly iPSC can also stimulate autoimmune reactions[11]. Indeed, long term culture, genomic instability, interference with matrix structure, genetic manipulation and epigenetic reprogramming can impair immune privilege status of the autologous cells. In the allogenic scenario, the expression of immune relevant molecules notably the polymorphic major histocompatibility complex (MHC) class I and II molecules (HLA class I and II in humans) is recognized to induce rejection. Human ESC express low level of HLA class I that significantly increases after differentiation[12] and expanding MSC *in vitro* remarkably increases their MHC II[13]. Beside the cell based immune rejection by cytotoxic T cells, another mechanism widely recognized as an important component of allograft failure in organ transplantation is antibody-mediated rejection (AMR)[14,15]. It results from the interaction of antibodies against mismatched donor antigens with the allograft vascular endothelium. Allosensitization to non-self highly polymorphic HLA is a major limitation of effective clinical organ, tissue, and cell transplantation. The worst-case scenario is when complement fixing IgG antibodies are present at the time of transplantation and these are directed to HLA class I, HLA-A and/or B antigens present in a donor tissue or organ (HLA-donor specific antibodies, HLA-DSA). In this case, an immediate immune reaction resulting in hyper-acute (HAR) or accelerated acute rejection is inevitable, and failure of the transplant through rejection of the graft is likely[14]. HLA-DSA activity may result in allograft injury through a variety of mechanisms, including both complement-dependent and independent pathways. While HLA molecules are known as antigen presenting structures, allowing a peptide to be recognized by the T cell receptors (TCR) in the context of self-MHC genetic restriction, evidence that HLA/MHC molecules are also bonafide signal transduction molecules is well documented and the biochemical pathways involved have been described[16,17]. This review discusses how the current knowledge and practical strategies developed in transplantation medicine can be translated to enable the development of safe personalized regenerative stem cell therapy.

MHC expression

The MHC class I antigen (HLA-A, -B, -C in humans), and the MHC class II (HLA-DR, -DQ, -DP in humans) are highly polymorphic cell membrane polypeptide chains. Most cells express MHC class I molecules. MHC class II molecules, in contrast, have a tissue-specific regulation of their expression, and their constitutive expression is practically restricted to antigen-presenting cells but also to endothelial cells. That most SCs express low MHC class I but not class II molecules brought the idea of those being immune privileged[18]. However, despite this low immunogenic profile *in vitro*, MSC and ESC trigger alloimmune response *in vivo*[9]. Porcine allogenic MSC are little or not immunogenic *in vitro* but their intracardiac injection elicited immune responses *in vivo*[19]. Allogenic murine ESCs also trigger cell infiltration and host immune response when injected in injured myocardium. These findings suggest that even if these cells initially lack the expression of immune relevant molecules such as MHC II, these may express these

molecules upon their administration *in vivo*. The stem cells operate within a microenvironment where these interact with stromal cells, growth factors, or extracellular matrix proteins and also face a variety of pro-inflammatory cytokines such as interferon γ and tumour necrosis factor alpha (IFNγ and TNFα). The microenvironment and its elements, together or independently, can modulate the expression of MHC on these cells[10]. When used in cell therapy, SCs are to be expanded *in vitro* often in medium supplemented with growth factors, which modifies the expression of several molecules. Human MSCs expanded *in vitro* in the presence of fibroblast growth factor (FGF) while retaining the conventional properties of MSCs, such as immunosuppression and multilineage differentiation, also express functional HLA-DR molecules and can present antigens. Simulation with IFNγ major regulator of MHC II, expression failed to induce the expression of HLA class II on human ESC[10]. However, evidence exists for the epigenetic control of MHC II and antigen processing molecules in human ESC and iPSC[20]. Epigenetic analysis showed that the regulatory regions of class II transactivator gene (CIITA) and HLA-DR genes are methylated in these cells and treatment with epigenetic agents restores both the expression of HLA II and its induction by IFNγ[10]. We also observed that IFNγ induces HLA II expression only in cardiac stem cells (CSC) maintained under hypoxic conditions and not under standard conditions[21]. These observations suggest that HLA II expression in CSC might be also regulated by epigenetic modifications provided by the hypoxic microenvironment. It seems clear today that SCs and their derived tissues express HLA I and can similarly to nearly all other cell type, be induced to express HLA II after transplantation when exposed to inflammatory cytokines or other microenvironment factors particularly during an intercurrent infection. Less is known concerning the expression of non-classical MHC class I (HLA-E, -G) and MHC class I-related antigens (MIC-A, MIC-B, *etc*.) on SCs. However, some evidence indicates that MSCs constitutively express HLA-G, and a functional role for this constitutive expression in mediating MSC immunosuppressive effect on T cell activation, proliferation, and/or natural killer (NK) and T cell-mediated cytotoxicity has been reported[22]. Both non-classical MHC class I and MHC class I-related antigens might be important issues for future considerations. In addition, non MHC immunogenetics may have additional impact on the fate of SC therapy[23].

Consequences of MHC expression

Alloreactive anti-HLA antibodies are generated by transfusion, pregnancy and organ or cell transplantation. Viral infections and vaccines can also induce a resurgence of these anti-HLA antibodies[24]. It is, therefore, likely that many potential recipients of SC therapy will have pre-existing anti-HLA antibodies. If donor specific, these antibodies may affect the SC in several ways. If cytotoxic these will limit the regenerative potential of the infused SCs. Alternatively these may modify the biology of the SC through their signalling capacity[10,18]. HLA antibodies are capable of inducing proliferation, maturation or apoptosis of different cells depending on their type and stage of differentiation[25-27]. Anti-HLA antibodies may contribute to the paracrine effect of the SC. This illustrates the possibility that anti-HLA antibodies can exert a detrimental as well as a beneficial effect in SC therapy. It is likely that a significant proportion of recipients would receive at the best partially HLA-mismatched SC-derived grafts given the necessity of "off-the-shelf" cells. Therefore, minimizing the risk while optimizing the benefit is mandatory for ultimate efficient regenerative therapy. What is an acceptable mismatching and how much risk is allowed and how much prophylactic immune protection is required will need to be investigated. Learning from present transplantation medicine will be, therefore, a fruitful approach in this context.

Transplantation immunology - point of view

In human organ transplantation, one approach to reduce graft immunogenicity is to minimize allogenic differences between donor and recipient by HLA matching. Ultimately, zero HLA-A, -B, -DR, -DQ mismatched transplants should be performed. Even without considering the existence of other important histocompatibility loci, this is extremely difficult to achieve due to extreme polymorphism of these MHC antigens and to the limited availability of fully matched donors. In the context of SC therapy, it is apparent that it would be similarly difficult. However, reducing HLA-mismatching by choosing the least immunogenic combination can now be achieved by banking stem cells.

The selection of the least incompatible SC with the host MHC is to be considered to avoid or attenuate the host immune response to the transplanted SC. One approach would be the development of HLA-matched cell banks. Knowing the extreme diversity of the

HLA system with an ever growing number of alleles (over 9000 at present)[28], it is not realistic to consider developing a fully matched HLA source of stem cells when the World Marrow Donor Registry, which includes at present over 20 x 10^6 individuals, covers only a fraction of the world HLA human diversity[29]. While a fully matched donor cell is not attainable, the possibility to select a donor cell from HLA "compatible" donor cell registry based on the frequency of the HLA haplotypes exists. The size and the optimal content of such a stem cell bank have been calculated based on the HLA haplotypes frequencies. Simulation programmes and mathematical models will be helpful in designing such cell banks to be economically sustainable. In this respect, it has been proposed to derive human ESCs and iPSCs from a restricted panel of HLA haplotypes, the use of HLA-A, -B, -DR homozygous donors may limit to below 100 number of haplotypes that would fit the requirement for most of the need of a heterogeneous population with the same ethnic background[30]. Therefore, the constitution of "haplobanks" among the large ethnic groups defined by their ancestry backgrounds may be relevant to the development of regenerative medicine[30]. The complete knowledge of the harmful and possible beneficial potential of anti-HLA antibodies in the context of SC therapy, is not yet attained. Therefore, it is mandatory to set up a "state-of-the-art" follow up in line with the current recommendations of both the American Society of Histocompatibility and Immunogenetics (ASHI)[31] and the European Federation of Immunogenetics (EFI)[32], in order to reach to an understanding of the implication of these antibodies in the course of SC therapeutics. Enhancing the awareness of SC transplantation teams to histocompatibility is probably the first action that can be followed by serious consideration of cost and management logistics. Recipients should be HLA typed for HLA-A, -B, -DR and -DQ, in the best achievable manner. HLA-C and HLA-DP typing can also be performed when recipient serum shows antibodies against these antigens. Screening for anti-HLA antibodies is started by a complete knowledge of the clinical history (previous sensitization) of the patient. Based on the protocols applied in the programmed organ transplantations, it is preferable to screen for anti-HLA antibodies at least twice pre-transplantation[10]. If delays in transplantation occur, testing can be done regularly with three months intervals but also two weeks and a month after any eventual sensitizing event. State-of-the-art assays include single-antigen Luminex® technology in combination with CDC (complement dependent cytotoxicity) assays. Despite its limits Luminex technology aims to identify anti-HLA antibodies with high specificity and sensitivity while CDC evaluates the cytotoxicity of dominant antibodies[10]. This knowledge will determine whether the patient is non-sensitized, immunized or hyperimmunized. Thus, an effective antibody detection should include its timing of occurrence, the immunoglobulin isotype – IgG or IgM, HLA-specificity – class I (HLA-A, -B, -C) or II (-DP, -DQ or –DP), and its strength or titre. The SCs to be administrated must be HLA typed and their DNA must be conserved for evaluation of other relevant immunogenetics such as cytokines, cytokine receptors and increate immunity molecules. It is now possible to determine the mismatches with the recipient and the eventual presence of DSA, using virtual cross-match. Any level of detectable donor specific anti HLA (DSA), IgG antibody pre-transplant is a risk in SC transplantation[33-36]. Conversely, identifying "permissive" versus "forbidden" mismatches can be a solution. Mismatches are more or less immunogenic. HLA matchmaker is a structure based matching prediction programme that considers each HLA antigen as a string of epitopes represented by short sequences of polymorphic amino acid residues in antibody exposed positions[37]. With this information, it is possible to transplant patients by simply avoiding forbidden mismatches[33]. Since the risk for antibody mediated rejection (AMR) and graft loss correlates with HLA-donor specific antibodies (DSA) peak and strength, the stratification of immunologic risk will help guide selection of acceptable grafts for sensitized patients. Similar to organ transplantation, to master the risk of HLA antibodies in SC therapy, algorithm based on pre-transplant single-antigen flow beads should be developed.

In spite of the above, techniques applied to identify the antibodies specificity using the cross-match approach limits and differences between *in vivo* and *in vitro* predicted reactivity can occur. Therefore, a cross-match assay is mandatory. Currently, two main complementary tests are available: complement-dependent cytotoxicity (CDC) and flow cytometry cross-match. However, conducting exactly the same tests in the context of SC transplantation is hardly attainable because lymphocytes from SC donors would be difficult to obtain. Results obtained by our group developing the assay for cardiac stem cell transplantation are encouraging (DG unpublished). Donor specific-antibodies against both HLA and non-HLA epitopes after administration of allogenic cells to

restore or repair various tissues have been identified. It is clear that as cellular therapies move to the clinic it will be essential to monitor recipients to establish whether they become sensitized and how sensitization influences the clinical course of the therapy[15]. Post-transplantation follow up can be also set up. *De novo* HLA-DSA could occur after organ transplantation and have been correlated with poor outcome[15]. Therefore, it is recommended to establish a post-transplantation systematic follow up monitoring the occurrence of HLA-DSA in serum taken from patients at predetermined appropriate times. In addition, regular follow up in case of suspicion of graft failure or rejection should be considered. These follow ups could be mainly conducted using single-antigen Luminex® technology. Also, the occurrence of *de novo* HLA antibodies after SC therapy may jeopardize the possibility for a patient to benefit later on of a heart transplant.

Concluding remarks

While at present the clinical benefit of SC therapeutics has been modest, some improvement on clinical end points have been reported. The use of allogenic SC products is attractive if these are highly standardized and available off the shelf. Therefore, for SC therapy to move to the clinics, immunological barriers should be overcome. To minimize immunogenetic differences between SC and recipient, detection of the immunization status of the recipient prior to SC injection, and monitor of both the allogenic and autoimmunity post-SC transplantation are to be recommended. The adaptation of the state-of-the-art assays that are currently used in organ transplantation is a logical step. Also, immunosuppression protocols should be considered. Integrating the unique immunobiology of SC with the patient immune status is the key to a successful translation of the SC therapy to the clinics.

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Reprint requests: Dr Dominique Charron, Laboratoire "Jean Dausset" - Laboratoire d'Immunologie et d'Histocompatibilité, Hôpital Saint Louis, CIB-HOG, AP-HP & INSERM UMRS 940, Institut Universitaire d'Hématologie, Université Paris-Diderot, 1, Avenue Claude Vellefaux, 75010, Paris, France
e-mail: dominique.charron@sls.aphp.fr