Humanized Mouse Model for Optimal Donor Selection in Transplantation

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ABSTRACT

The outcome of organ transplantation is largely dictated by selection of a well-matched donor, which results in less chance of graft rejection. An allogeneic immune response is the main immunological barrier for successful organ transplantation. Donor and recipient human leukocyte antigen (HLA) mismatching diminishes outcomes after solid organ transplantation. The current evaluation of HLA incompatibility does not provide information on the immunogenicity of individual HLA mismatches and impact of non-HLA-related alloantigens, especially in vivo. Here we demonstrate a new method for analysis of alloimmune responsiveness between donor and recipient in vivo by introducing a humanized mouse model. Using molecular, cellular, and genomic analyses, we demonstrated that a recipient's personalized humanized mouse provided the most sensitive assessment of allogeneic responsiveness to potential donors. In our study, HLA typing provided a better recipient-donor match for one donor among two related donors. In contrast, assessment of an allogeneic response by mixed lymphocyte reaction (MLR) was indistinguishable between these donors. We determined that, in the recipient's humanized mouse model, the donor selected by HLA typing induced the strongest allogeneic response with markedly increased allograft rejection markers, including activated cytotoxic Granzyme B-expressing CD8+ T cells. Moreover, the same donor induced stronger upregulation of genes involved in the allograft rejection pathway as determined by transcriptome analysis of isolated human CD45+cells. Thus, the humanized mouse model determined the lowest degree of recipient-donor alloimmune response, allowing for better selection of donor and minimized immunological risk of allograft rejection in organ transplantation. In addition, this approach could be used to evaluate the level of alloresponse in allogeneic cell-based therapies that include cell products derived from pluripotent embryonic stem cells or adult stem cells, both undifferentiated and differentiated, all of which will produce allogeneic immune responses.

INTRODUCTION

Solid organ transplantation has been a life-saving procedure for thousands of patients worldwide. Recent advances on improving donor-screening diagnostics have aimed at identification of the most compatible donor for the transplant recipient to maximize allograft survival. Current standards of donor selection rely on HLA typing and in vitro MLR, which does not take into account the in vivo environment and recipient's adaptive immune response. Humanized mouse models are an appealing alternative that permits personalized investigation of the immunocompatibility of potential donor tissues for the recipient human immune system without putting patients at risk. By utilizing genomics, molecular and cellular analyses of allogeneic immune response we analyze the efficiency of our novel humanized mouse model to assess the donor-recipient compatibility and determined that it to be significantly more sensitive than conventional screening methods.

METHODS

HLA typing and MLR for histocompatibility. Special strain of immunodeficient mice, NSG mice, subjected to irradiation (2Gy) and i.v injection of 8 × 10 peripheral blood mononuclear cells (PBMCs) from transplant recipient. For allogeneic immune response, humanized mouse received 3 × 10 PBMCs from unrelated donors(UD) or related donors(RD). Whole genome transcriptome analysis and Real-Time PCR (RT-PCR) Transplant Rejection Array was used.

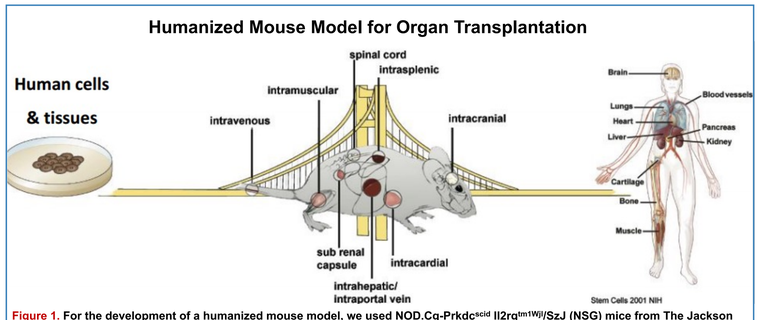

Figure 1. For the development of a humanized mouse model, we used NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice from The Jackson Laboratory. NSG mice (5 to 12 weeks old) were given a single intravenous lateral tail injection of different amounts of human peripheral blood mononuclear cells (PBMCs) from healthy volunteers (recipients).

RESULTS

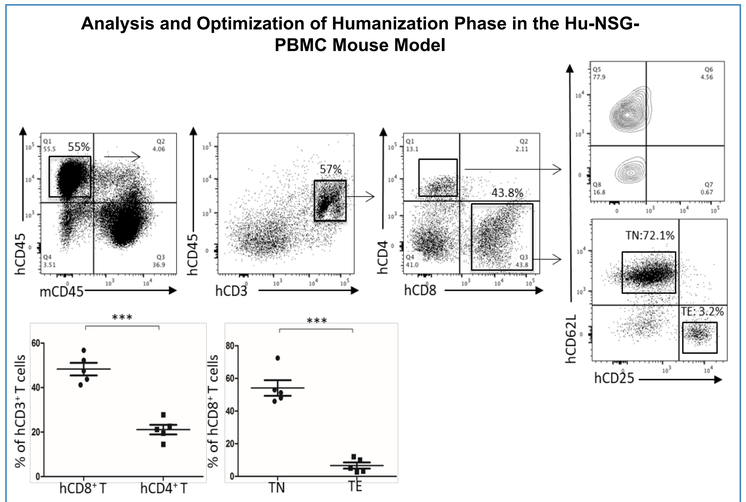

Figure 2. Flow cytometry dot plots depict phenotype of the engrafted hCD45$^+$ cells in the humanized mouse with gating for hCD3$^+$, hCD4$^+$, and hCD8$^+$ T cell populations. Characterization of hCD4 and hCD8$^+$ T cells was expanded showing their respective CD62L and CD25 expression. Graphical summary depicts frequency (%) of hCD4 and hCD8$^+$ T cells as well as percent of CD62L$^+$CD25$^-$ and CD62L$^-$CD25$^+$ populations. N=5 mice per group. Data presented as mean ± SD. ***p <0.001, ****p<0.0001.

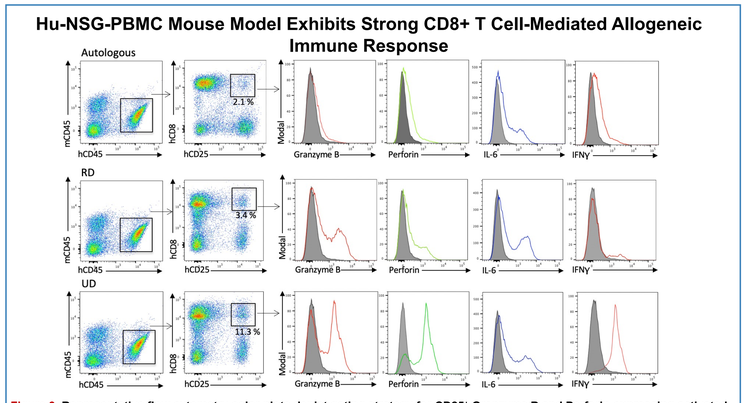

Figure 3. Representative flow cytometry color plots depict gating strategy for CD25$^+$ Granzyme B and Perforin-expressing activated cytotoxic hCD8$^+$ T cells in spleen of the humanized mouse for the Autologous, Related Donor (RD), and Unrelated Donor (UD) challenge groups. Histogram shows expression of pro-inflammatory cytokines IL-2 (red line) and IFN-γ (blue line) amongst these cytotoxic hCD8$^+$T cells.

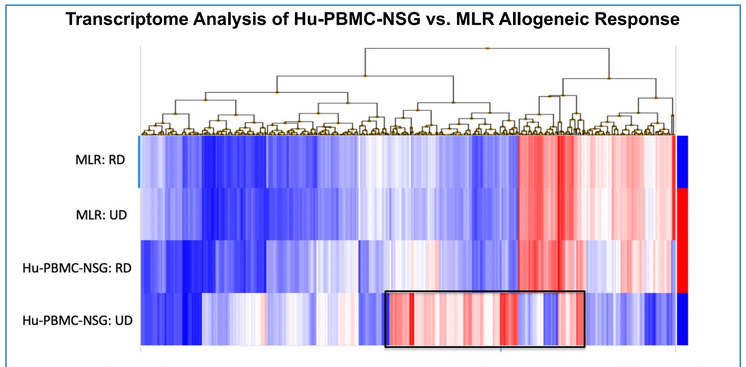

Figure 4. Human Gene 2.0 ST array was used to plot a heat map representing differential gene expression in hCD3$^+$ T cells of the allogeneic RD and UD challenges for the MLR and the humanized mouse models. Hierarchical clustering was used to segregate individual gene expression in each group into reduced expression (blue) and over-expression (red) conditions.

SUMMARY

Immunological graft rejection originates from alloimmune T cells derived from the host immune system. The host alloimmune T cell response against donor-derived antigens such as HLA are much stronger in comparison to classical immune responses against pathogen or self. This is due to the highly polymorphic nature of HLAs combined with the presence of multiple HLA loci (HLA-A, B-class I antigens; HLA-DR, DQ, DP- class II antigens). A diverse mismatch across the HLA alloantigens between the recipient and donor can elicit a robust allogeneic immune response. These allospecific T cells of the host immune system can form activated cytotoxic Granzyme B-expressing CD8$^+$ T cells that are primarily responsible for graft tissue destruction and transplant failure. Therefore, selection of the most immunocompatible donor for the recipient with the least amount of HLA mismatches can maximize graft survival and reduce dependency on immunosuppressive treatments. In this scenario, a well-matched related donor is the perfect candidate, allowing adequate time for investigating the level of histocompatibility with the recipient. HLA typing and MLR are the long-time clinical standards for the assessment of donor-recipient immunocompatibility in organ transplantation. Various studies have established that an increased number of matched antigens and decreased number of mismatched antigens lead to improved graft survival. However, HLA typing results are often confounded by the varying immunogenicity of the different HLA loci. HLA-DR mismatches are known to contribute heavily to graft rejection, and HLA-A and B matches are also crucial for graft acceptance. Meanwhile, HLA-DQ mismatch has no clinical significance unless it is compounded by the presence of a DR mismatch; DP mismatches only become relevant during regraft scenarios. Current donor organ allocation strategies consider mismatches at HLA-A, B, and DR to be equally important. However, mounting evidence suggests that each HLA mismatch contributes differently to graft survival; some HLA mismatches look more permissible than others. Benefits of dependence on HLA matching are further diluted by other factors such as age; a younger donor age can compensate for the impact of HLA mismatches. However, HLA typing does not consider the impact of non-HLA-related alloantigens. Growing evidence suggests that as much as 38% of kidney allograft rejections are due to these non-HLA-related alloantigens in comparison to 18 % caused by HLA mismatches. Thus, there is a need for additional methods to assess immunocompatibility between recipient and donor. The most common and routinely used solution is MLR. CFSE-based MLR assays enabled the phenotypic characterization of alloimmune T cells developed by the recipient on stimulation by donor immune cells. However, the correlation between clinical outcomes and *in vitro* functional MLR assays has been low due to the inherent inability of *in vitro* assays to replicate the *in vivo* physiological environment. For example, unresponsive donor reactive cells commonly seen in MLR may arise due to deletion or anergy, a phenomenon that is indistinguishable in an *in vitro* setting.

OBSERVATIONS

Our findings demonstrate:

1.) Hu-PBMC-NSG mouse show efficient engraftment with up to 60% of the engrafted CD8$^+$ T cells being naïve T cell (TN) phenotype being CD62L$^+$CD25$^-$

2.) The NSG-PBMC model showed a selective immune CD8+ T cell-based response on the UD allogeneic stimuli while at the same time exhibited a tempered/controlled feedback to the RD challenge

3.) The NSG-PBMC model showed an immune transcriptional profile

4.) MLR expresses minimal allogeneic response both cellular and transcriptional

5.) The Hu-PBMC-NSG model is significantly more sensitive than MLR

CONCLUSION

NSG-PBMC humanized mouse model was able to identify the related donor exhibiting minimal allogeneic response to the recipient. This model is significantly more immunologically sensitive than conventional MLR and HLA typing for selection of an immunocompatible donor for the transplant recipient.

ACKNOWLEDGEMENTS

This study was supported by research funding from the Carlos and Marguerite Mason Trust, and by the U.S. National Institute of Health, National Cancer Center Institute Grant CA 172230. I would also like to thank members of the Horuzsko Lab and the Division of Nephrology at the Medical College of Georgia.