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Dynamical System Modeling to Simulate Donor T Cell Response to Whole Exome Sequencing-Derived Recipient Peptides Demonstrates Different Alloreactivity Potential in HLA-Matched and -Mismatched Donor–Recipient Pairs

Badar Abdul Razzaq [1], Allison Scalora [2], Vishal N. Koparde [3], Jeremy Meier [2], Musa Mahmood [1], Salman Salman [1], Max Jameson-Lee [2], Myrna G. Serrano [3], Nihar Sheth [3], Mark Voelkner [4], David J. Kobulnicky [2], Catherine H. Roberts [2], Andrea Ferreira-Gonzalez [4], Masoud H. Manjili [5], Gregory A. Buck [3], Michael C. Neale [6], Amir A. Toor [2,*]

[1] *Virginia Commonwealth University School of Engineering, Virginia Commonwealth University, Richmond, VA 23298*
[2] *Bone Marrow Transplant Program, Massey Cancer Center & Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA 23298*
[3] *Center for Biological Complexity, Virginia Commonwealth University, Richmond, VA 23298*
[4] *Department of Pathology, Virginia Commonwealth University, Richmond, VA 23298*
[5] *Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298*
[6] *Department of Psychiatry and Statistical Genomics, Virginia Commonwealth University, Richmond, VA 23298*

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Immune reconstitution kinetics and subsequent clinical outcomes in HLA-matched recipients of allogeneic stem cell transplantation (SCT) are variable and difficult to predict. Considering SCT as a dynamical system may allow sequence differences across the exomes of the transplant donors and recipients to be used to *simulate* an alloreactive T cell response, which may allow better clinical outcome prediction. To accomplish this, whole exome sequencing was performed on 34 HLA-matched SCT donor–recipient pairs (DRPs) and the nucleotide sequence differences translated to peptides. The binding affinity of the peptides to the relevant HLA in each DRP was determined. The resulting array of peptide-HLA binding affinity values in each patient was considered as an *operator* modifying a hypothetical T cell repertoire *vector*, in which each T cell clone proliferates in accordance with the logistic equation of growth. Using an iterating system of matrices, each simulated T cell clone's growth was calculated with the steady-state population being proportional to the magnitude of the binding affinity of the driving HLA–peptide complex. Incorporating competition between T cell clones responding to different HLA–peptide complexes reproduces a number of features of clinically observed T cell clonal repertoire in the simulated repertoire, including sigmoidal growth kinetics of individual T cell clones and overall repertoire, Power Law clonal frequency distribution, increase in repertoire complexity over time with increasing clonal diversity, and alteration of clonal dominance when a different antigen array is encountered, such as in SCT. The simulated, alloreactive T cell repertoire was markedly different in HLA-matched DRPs. The patterns were differentiated by rate of growth and steady-state magnitude of the simulated T cell repertoire and demonstrate a possible correlation with survival. In conclusion, exome wide sequence differences in DRPs may allow simulation of donor alloreactive T cell response to recipient antigens and may provide a quantitative basis for refining donor selection and titration of immunosuppression after SCT.

INTRODUCTION

Stem cell transplantation (SCT) from HLA-matched donors delivers curative therapy to patients with hematologic malignancies, but at the cost of significant morbidity derived from the alloreactive phenomenon of graft-versus-host disease (GVHD) and the immunosuppression administered to

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* Correspondence and reprint requests: Amir A. Toor, MD, Associate Professor of Medicine, Bone Marrow Transplant Program, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298.
E-mail address: amir.toor@vcuhealth.org (A.A. Toor).

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control it [1-3]. Immune reconstitution after allografting is critical for relapse prevention and survival but may be associated with a higher risk of GVHD in some individuals [4,5]. Because of the complexity of the variables involved, clinical outcomes after SCT have been studied as a probability function of various clinical features of the transplant. Donor type, histocompatibility, transplant donor–recipient gender matching, minor histocompatibility antigens (miHAs), and intensity of immunosuppression before and after transplantation have figured prominently in such analyses [6-8]. However, within the constraints of these parameters, it is still difficult to precisely predict the development of alloreactivity in *individuals* undergoing SCT [9]. Further, relatively simple changes in immunosuppressive regimens (to neutralize HLA-mismatch–directed alloreactivity) have allowed transplantation to be performed using donors with increasing levels of disparity across the HLA loci, best exemplified by related, HLA-haploidentical SCT and unrelated, umbilical cord blood transplantation [10]. In these examples of HLA-mismatched transplants, GVHD rates are similar or lower when compared with HLA-identical donors. Although these differences are partly a function of the immunosuppressive regimens used and partly due to the lack of a mature T cell repertoire, they point to the possibility that there does *not* exist a *linear* quantitative relationship between the extent of HLA matching in transplant recipients and donors and clinical outcomes. This observation and the apparently random nature of occurrence of alloreactivity in similarly HLA-matched (or -mismatched) individuals suggests that genetic disparity across other regions of the exome (as the determinant of miHA) has a major influence on the clinical outcomes.

From an immunologic standpoint, *alloreactivity* in recipients of HLA-matched SCT is driven in part by histoincompatibilities between transplants donors and recipients. In patients matched for the MHC loci, these miHA generally provide the trigger for initiation of alloreactive tissue injury by donor T cells. A simple whole exome sequencing (WES) approach has demonstrated large numbers of potentially immunogenic DNA sequence differences between transplant donors and recipients [11]. These exonic DNA sequence differences can be analyzed to derive amino acid sequences that yield different peptides, which in turn demonstrate a range of binding affinities for the HLA molecules present in a specific donor and recipient. This WES + *in silico* HLA-peptide binding affinity procedure yields a large donor–recipient pair (DRP)-specific library of putative miHA that may contribute to alloreactivity, resulting in GVHD (HLA binding peptides absent in donors but present in recipients) [12]. Compared with the experimentally determined miHA, this computational procedure yields several hundred to thousands of *potential miHAs* in each HLA-matched DRP and thus introduces a very large set of variables that cumulatively may influence the likelihood of GVHD incidence in individual SCT recipients. This *derived* variant HLA binding peptide library does not reflect the *entire* histocompatibility antigen spectrum; however, it gives an *estimate* of the scale of *antigen diversity* encountered by the donor T cells when transplanted into the recipient and may be considered as an *estimated alloreactivity potential*. This measure of antigenic diversity may then be studied to lend an insight into the T cell response to the miHA library in each patient.

The large array of potential peptide targets of alloreactivity, each with a different binding affinity to HLA, is presented on a limited number of HLA class I (present endogenous antigens) and HLA class II (present exogenous antigens) molecules in the recipient tissues. This makes it very unlikely that GVHD incidence is a probability function of a small number of critical variables; rather, it is more likely that GVHD is the result of an integrated sum of all the variant immunogenic peptides presented by the HLA in a unique DRP, modulated by the immunosuppressive influence of pharmacotherapy during immune reconstitution. This is a logical assumption because donor T cell reconstitution corresponds with the likelihood of alloreactivity developing [4,13,14]. To develop a model to estimate the likelihood of alloreactivity developing in unique SCT DRPs, immune response to the peptide–HLA complex library will need to be modeled in each individual. In such a model the binding affinity of the miHA to the relevant HLA molecules will be one of the determinants of the likelihood of individual miHAs being presented to T cells.

T cell repertoire after SCT comprises thousands of T cell clones, which span a large range of clonal frequencies, with a different range of frequencies for different T cell clones observed in each individual. Over the lifetime of an individual, a self-tolerant, pathogen-responsive set of T cell clones is developed that responds to pathogen-derived peptides in the context of an individual's HLA molecules. The TCRs characterizing these clones are coupled with CD3 molecules, which deliver a proliferation/activation signal to the T cells when the *first signal*, target antigen–HLA complex, is encountered. Therefore, depending on the presence and immunogenicity of the target antigens, T cell clones may be abundant or scarce. An important moderator of the immune responsiveness is the presence of the *second signal*, which either enhances or extinguishes immune responsiveness (CD28 and PD-1, respectively). Each functional T cell subset possesses a unique array of TCR αβ and can recognize and respond to a unique array of HLA–peptide complexes. The eventual T cell repertoire is massive, potentially numbering in the several hundred thousand to more than a million unique T cell clones [15-17]. High throughput sequencing of TCR cDNA obtained from isolated T cells demonstrates that the distribution of T cell clonal frequency when ordered according to clonal rank conforms to a Power Law distribution, declining as a power of the number of clones examined [18-21]. These clonal frequencies may change under different conditions, such as upon encountering pathogens in normal circumstances or in SCT upon encountering alloreactive antigens. Specifically, after SCT the T cell clonal repertoire in the donor product is altered, with clones that were once dominant becoming suppressed and other previously repressed clones becoming ascendant [18,22].

To more accurately compute the likelihood of alloreactivity developing in individual DRPs, it is imperative that the interaction between the parallel systems of antigen presentation and T cell response be modeled quantitatively. Lymphocyte reconstitution after SCT has been modeled as a *logistic dynamical system*, with a familiar sigmoid growth pattern over time (Supplementary Figure 1) [4,23]. Logically then, various T cell subsets, including T cell clones, should demonstrate the same growth dynamic. This makes it possible to develop a model, which may predict immune reconstitution after SCT, by simulating the T cell repertoire emerging in response to the miHA–HLA complex library derived from DRP exome differences (Appendix).

In this article we present a model of T cell reconstitution based on the application of the logistic dynamical system to a hypothetical T cell repertoire responding to the