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Expanding the toolkit for the study of allo-specific B and T cell responses

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Immune responses to the allograft by both B and T lymphocytes cause significant complications for long-term graft survival after transplantation. This is an exciting time as new tools and assays that enable us to define and study these responses are being developed.

In the current issue of Transplantation, Young et al,[1] review a variety of methods that can be used to detect alloreactive B and T cells and place the methods in the context of the knowledge gained and potential future applications. Some of the methods will be familiar to readers of Transplantation as they have been used extensively in studies of transplantation, such as mixed lymphocyte reactions (MLR), in vivo mouse models including model antigens and/or T cells with transgenic (Tg) T cell receptors (TCRs), trans-vivo delayed type hypersensitivity, and quantification of donor-specific antibody. Others have only recently been applied to transplantation, such as intravital imaging, in vivo tracking of immune cells, and use of major histocompatibility complex (MHC) multimers. And several additional methods have yet to be applied to the study of transplantation but show significant promise in this area. These techniques include barcoded multimers with a variety of MHC specificities,[2] reversible MHC multimers, and retrogenic TCRs.[3] Each of these methods brings new opportunities for studying alloreactive cells at the single cell level. This valuable resource covers significant breadth in the methodologies available to study alloimmunity, including the advantages and disadvantages of each method.

A crucial factor in the study of allograft recognition by T cells is the unique nature of alloantigen presentation. T cells can respond directly to MHC presented on donor antigen presenting cells, or indirectly to processed donor antigen on recipient antigen presenting cells. Most in vitro and in vivo assays analyze primarily direct presentation, but several approaches are described to study the indirect pathway. In vivo, either direct or indirect pathways can be studied with Tg TCRs restricted to recognition of donor or recipient MHC or a specific peptide-MHC, respectively. Young et al, have included a table describing various TCR Tg mice that have been used in the study of alloreactivity,[1] which is a valuable

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resource for researchers contemplating studies of the alloimmune response. Another promising technology is retrogenic TCRs,[3] which are retrovirus expressing designated TCR rearrangements that can be used to transduce bone marrow progenitors. Mouse models with retrogenic TCRs can be generated more rapidly than TCR Tg mice and can be used for analysis of multiple alloreactive TCRs. Analyses using TCRs with known, defined allo-specificity have the benefit of potential in vivo imaging to track T cell migration, providing insight into the mechanism of allograft reactive T cell activation and migration to the allograft.

While genetically modified mice offer an approach to mechanistic study, totally different approaches are needed to study the human immune response to allografts at the single cell level. A significant limitation in the field of alloimmunity is the difficulty of defining the alloantigen that is the target of the immune response. This difficulty arises both from the diversity of alloantigen, and from the need to link the B or T cell antigen receptor to the specific antigen. A variety of methods to address this limitation are discussed in this review. The most well-studied are the use of mouse models with defined alloantigen, and in some cases Tg alloreactive TCRs, or the use of patient samples of a very limited range of Human Leukocyte Antigen (HLA) types. However, given the vast diversity of HLAs in the human population,[4] these methods only address a subset of possible antigen sources. Novel methods to increase the throughput of analysis of alloimmunity for both T cells and B cells are also clearly described and referenced. One exciting example is a recently developed method that involves the use of a panel of up to 1000 different peptide-MHC multimers to stain and sort T cells.[2] The individual specificities are identified through distinct barcodes on each multimer, and thus this method can detect a wide range of responses to HLA and other antigens. And MHC multimers are not limited to the study of antigen specific T cells. MHC multimer methods that identify MHC-specific B cells can identify alloantigen-specific B cells, track them during an allo-response and will allow a significantly improved understanding of antibody-mediated rejection. Non-HLA antibodies and B cells reactive for multiple antigens can be detected by antibody binding to an array of proteins, or lysate of apoptotic cells. Antibodies to HLA can be detected with a high throughput assay in which microspheres are coated with different HLA molecules, and B cell binding to each type of microsphere detected by flow cytometry.[5] These newer methods hold promise to provide a great deal of further insight into alloreactive B cells and potentially improve the accuracy of diagnostic tests.

The limitations of each assay are important to consider in selection of a method to analyze allograft reactivity. In vitro assays, such as MLR and ELiSPOT (enzyme linked immunospot), have been extremely effective in identifying alloreactive T cells. However, these methods do not reflect the kinetics of in vivo responses to alloantigen or the complexities of cell to cell interactions and cytokine milieu in the in vivo environment. In vivo approaches including adoptive transfer and footpad injection in mice can address limitations of in vitro assays. Combination approaches are discussed and must be employed to fully study underlying mechanisms of alloimmune responses.

The most promising aspect of this review is the potential for applications of new technologies. While immunosuppression is quite effective in preventing T cell mediated

rejection, it is less effective in preventing antibody-mediated rejection.[6] In particular, advances in the study of antigen specific B and T cells allow tracking of cell populations in vivo, which provides insight into activation, differentiation, and migration. High throughput analyses of antigen specificity will finetune our knowledge of the mechanisms of allograft rejection mediated by both cell types. Readers of Young et al, will be fully versed in conventional and emerging technologies that can be used to gain a mechanistic understanding of alloreactivity and graft rejection both in animal models and humans, and hold promise for development of diagnostics to improve graft survival in the clinic.

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Abbreviations

HLA	human leukocyte antigen
MHC	major histocompatibility complex
MLR	mixed lymphocyte reaction
TCR	T cell receptor
Tg	transgenic

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