ArrayPlate method (High Throughput Genomics (HTG), Tuscon, AZ) enables gene expression measurement without the need for RNA isolation, reverse transcription, or amplification, each of which are technically difficult in FFPET and in combination can lead to confounding results, while the use of short 50-mer probes enables hybridization, despite RNA fragmentation, usually to fragments approximately 200bp long. It is intriguing that the majority of the prognostic genes were unchanged between the pre-rituximab and rituximab treatment groups, in contrast to some other studies, but this study investigated a wide range of genes from several other studies and this may have increased the possibility of identifying a common set of genes, while the ArrayPlate method captures all mRNAs without RNA extraction by the use of short probes capable of hybridization to both soluble and insoluble/cross-linked mRNA of importance in FFPET. Adapted by permission from Macmillan Publishers. Roberts RA, Sabalos CM, LeBlanc ML, et al. Quantitative nuclease protection assay in paraffin-embedded tissue replicates prognostic microarray gene expression in diffuse large-B-cell lymphoma. Lab Invest. 2007;87:979-997.

Comment on Taylor et al, page 3508

Donor dendritic cells dance the do-si-do in allogeneic transplantation

Edmund K. Waller and Jian-Ming Li
EMORY UNIVERSITY

In this issue of Blood, Taylor and colleagues studied the effect of CpG oligonucleotide ligands for TLR9 on host and donor APCs in a murine model of allogeneic bone marrow transplantation. The article describes an unexpected result: administration of CpG activated both donor and host DCs, resulting in enhanced rates of graft rejection and accelerated GVHD.

The current paradigm for understanding engraftment in allogeneic hematopoietic progenitor cell transplantation is that donor T cells facilitate engraftment by donor stem cells, host dendritic cells (DCs) activate donor T cells and induce graft versus host disease (GVHD), and host T cells mediate graft rejection. The role for donor DCs has been neglected due, in part, to their scarcity in bone marrow grafts, assumptions of limited survival in allogeneic recipients after transplantation, and the overwhelming larger numbers of host DCs that are capable of directly presenting alloantigen to donor T cells. A novel finding in the report by Taylor et al relates to a role for donor DCs in modulating engraftment. The study is also of some interest to transplant immunologists, since it suggests caution in using method as having “the potential to change the practice of pathology.” A masterful use of the understatement!

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

nonspecific immune adjuvants due to the increased risks of deleterious host-initiated and donor-initiated innate and adaptive immune responses. Administration of Cpgoligonucleotides resulted in increased infiltration of lymphoid organs and Peyer patches by donor T cells in allogeneic recipients. The augmented alloreactivity was made manifest as increased GVHD and graft rejection, with the donor and host T cells activated by host-type and donor-type antigen-presenting cells (APCs), respectively, following toll-like receptor 9 (TLR9) ligand binding to APCs. Accelerated GVHD (but not graft rejection) was dependent on IFN-γ synthesis by T cells. Of note, bone marrow from donor mice deficient in CD80 or CD86 costimulatory molecules engrafted more efficiently after recipients received sublethal irradiation, supporting the role for donor APCs in activating host immunity and effector T cells that mediate graft rejection.

The paper by Taylor et al represents part of an emerging paradigm that runs counter to existing dogma regarding the importance of host DCs rather than donor DCs in allogeneic transplantation. This report represents the first clear demonstration of an important effect of donor DCs, contained in the graft, in the regulation of host immune activation. Complementary studies from our own group have demonstrated a role for donor DCs in augmenting the alloreactivity of donor T cells and enhancing graft versus leukemia effects. Thus, it is time for donor DCs to take their turn in the dance of cell types involved in allogeneic transplantation. These donor cell populations are excellent targets for novel maneuvers in graft engineering designed to reduce graft rejection by reducing host T-cell activation or augment donor T-cell alloreactivity to increase graft versus leukemia effects.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES

Comment on Friedman et al, page 3517

Raising the spectra of T-cell profiling

William J. Murphy UNIVERSITY OF NEVADA

Friedman and colleagues use spectratyping to identify donor T cells that respond to host minor histocompatibility antigens in vitro and in patients after allogeneic HSCT.

The use and efficacy of allogeneic hematopoietic stem cell transplantation (HSCT) has been severely hampered by the occurrence of graft-versus-host disease (GVHD), in which donor T cells attack the genetically disparate host. Separating the T cells that mediate GVHD from those that produce beneficial graft-versus-tumor (GVT) effects and/or provide resistance to opportunistic infections has remained the Holy Grail of allogeneic HSCT. In this issue of Blood, Friedman et al suggest that a new era may be coming whereby T-cell receptor (TCR) profiling using spectratyping would allow for analysis of different T-cell populations in the donor that can ultimately respond to host minor histocompatibility antigens (miHA) and mediate GVHD. Spectratyping may allow for better donor selection as well as a means to identify and then remove deleterious T-cell populations from donor grafts.

Allogeneic HSCT offers tremendous potential benefits for cancer therapy due to the existence of GVT responses by donor T cells that can help eradicate the tumor. Unfortunately, some of these donor T cells may also mediate deleterious GVHD, which significantly limits the efficacy and application of this procedure. Early attempts at graft engineering had nonselectively removed the donor T cells from the graft, which reduced GVHD occurrence, but also obviated the GVT effects and led to increased graft rejection as well as increased susceptibility to opportunistic infections. There has been tremendous interest in finding a means to specifically remove the GVHD-inducing donor T cells from the graft but allow other T cells to remain and perform their useful functions. The question is how to identify the “bad” T cells? Recent studies have focused on a number of different ways to transfer “safer” T cells via separation based on differences including CD4+ T-cell subsets (Th2, Tregs), naive phenotype, or activation marker expression after mixed lymphocyte culture (MLC) assays as reviewed by Welniak et al. The primary problem is that all these methods are inefficient, GVHD-mediating cells may be missed, and GVT-producing cells may be adversely targeted. Adding to the complexity, numerous investigators tried to correlate in vitro MLC proliferation assays or cytotoxic T-cell precursor frequencies with GVHD to help choose and determine optimal donors. While in principle these attempts...
Donor dendritic cells dance the do-si-do in allogeneic transplantation

Edmund K. Waller and Jian-Ming Li