Alternative mechanisms that mediate graft-versus-host disease in allogeneic hematopoietic cell transplants

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Allogeneic hematopoietic cell transplantation (alloHCT) benefits increasing numbers of patients with otherwise lethal diseases. Graft-versus-host disease (GVHD), however, remains one of the most potentially life-threatening complications due to its own comorbidities and the side effects of its treatment. In this issue of the *JCI*, two groups have turned dogma on its head by providing evidence for alternative mechanisms of acute GVHD (aGVHD) in humans. The principle of donor T cell reactivity elicited by host antigen-presenting cells (APCs) expressing MHC-encoded major HLA disparities or expressing minor histocompatibility antigen (miHA) differences presented by identical HLA molecules remains intact. These reports, however, demonstrate that GVHD can additionally result from peripheral host T cells resident in skin and gut being stimulated against donor APCs in the form of monocyte-derived macrophages. Moreover, these donor monocyte-derived macrophages can themselves mediate cytopathic effects against resident host T cells in skin explants and against a keratinocyte-derived cell line.

Introduction

Hematopoietic cell transplantation (HCT) has its greatest application in treating hematologic and lymphoid malignancies, but also some solid tumors and selected autoimmune diseases (1). Malignant diseases most responsive to HCT are those in which patients achieve remission after standard therapy. HCT prolongs that remission and often achieves long-term cures.

Approximately 40% of HCTs are allogeneic (alloHCT), with autologous transplants (autoHCT) comprising the remainder (1). Molecular typing of human leukocyte antigens (HLA) and killer immunoglobulin-like receptors (KIR) has improved donor selection using stem cells from bone marrow, granulocyte colony-stimulating factor–elicited (G-CSF–elicited) peripheral blood stem cells, or umbilical cord blood. Improved strategies to prevent graft-versus-host disease (GvHD) and prevent or cure opportunistic infections have also facilitated broader and more successful therapeutic use of alloHCT.

GVHD can nevertheless still develop in two situations. The first and most obvious occurs when T cells recognize and react against mismatches between host and donor HLA molecules, encoded by codominant genes comprising the major histocompatibility complex (MHC). The second results from T cells' encountering minor histocompatibility antigen (miHA) disparities derived from nucleotide polymorphisms, but presented and recognized in the context of MHC identity between HLA-matched host-donor pairs (2). GVHD occurs in both acute and chronic forms, and patients can suffer from one or the other or both. Common wisdom views donor T cells as the predominant lymphocyte mediators of acute GVHD (aGVHD), whereas dysfunctional donor B cells underlie the development of chronic GVHD (cGVHD).

Prior state of the art

Investigators have used rodent models to understand alloHCT and its complications, but there are important differences from what occurs in humans. Mouse models employ inbred strains to control or limit experimental variables, whereas the human population is outbred. Mouse bone marrow is also harvested by flushing marrow directly from long bones, with little to no contamination by circulating T cells. GVHD in mice transplanted with allogeneic bone marrow therefore requires the addition of donor T cells, typically from spleen. In fact, it was in the late 1970s that Korngold and Sprent first published that addition of mature T cells caused a dose-dependent increase in severity and eventual lethality of GVHD, mediated solely by minor histocompatibility differences (3). Since then, investigators have designed mouse models under the presumption that donor T cells are necessary and sufficient for aGVHD. Moreover, GVHD mouse models also use naive mice housed in specific pathogen–free facilities. These mice thereby lack memory T cells with diverse repertoires, especially resident populations in tissues.

Efforts to reduce or eliminate GVHD in humans have therefore focused on interfering with T cell function either pharmacologically or physically. Pharmacologic GVHD prophylaxis impairs activation and

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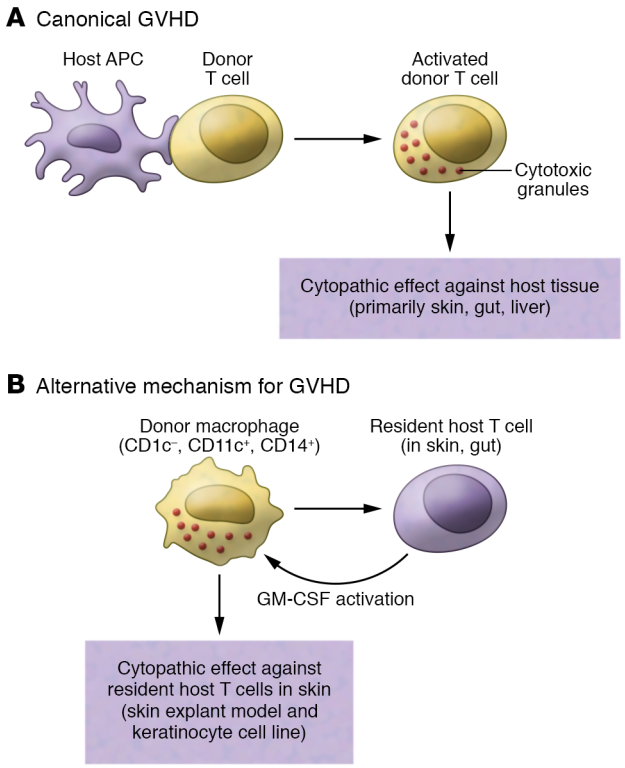

Figure 1. Canonical versus alternative GVHD model. (**A**) In canonical GVHD, host APCs stimulate donor T cells, which in turn attack host cells in tissue (primarily in the skin, gut, and liver). (**B**) The alternative GVHD mechanism, supported by data from Divito et al. and Jardine et al. (9, 10), results from resident host memory T cells' being stimulated by inflammatory $CD1c^{neg}$, $CD11c^{pos}$, $CD14^{pos}$ donor–derived macrophages in the skin and gut in what amounts to HVG reactivity that is clinically indistinguishable from GVHD. In this alternative GVHD mechanism, inflammatory donor-derived macrophages also mediate a direct cytopathic effect against resident host T cells in skin explants and against a keratinocyte-derived cell line.

expansion of alloreactive T cells, though it can also interfere with T cell responses against pathogens. Negative selection of hT cells or positive enrichment of $CD34^+$ progenitors can also physically deplete T cells from the allograft (4). More recently, there is greater interest in the use of post-transplant cyclophosphamide, which targets donor T cells newly reactive against host alloantigens (5), but spares engrafting progenitor populations, beneficial Tregs (6), and other populations that possess aldehyde dehydrogenase (ALDH) activity to resist cyclophosphamide (6).

An immunologic process that overlaps with but is distinct from GVHD is termed graft-versus-leukemia/lymphoma (GVL) or graft versus tumor (GVT) (7, 8). Here, donor T cells sensitized against tumor-derived miHA and presented in the context of HLA identity can mediate cytotoxicity against those tumor cells without necessarily also targeting normal host tissue. GVL/GVT can even occur when allografts are initially depleted of T cells, hence relying on engrafting donor T cells that acquire reactivity against miHAs expressed by the malignancy. Intact T cell populations in the allograft are more essential for mediating GVL in patients undergoing transplant for refractory disease, and there may be greater overlap with GVHD. In contrast, patients transplanted in remission because of high relapse risk can benefit from GVL mediated by engrafting donor T cells without developing GVHD. The bottom line is that the requirement for engrafting to intact T cell effectors in the initial allograft moves along a continuum from remission to refractory disease with regard to mediating GVL/GVT against the malignancy for which allotransplant is undertaken.

Graft-versus-host, host-versus-graft, or both?

In this issue of the *JCI*, Divito et al. and Jardine et al. (9, 10) focus exclusively on human alloHCT because of the above noted limitations of mouse allotransplant models. Each group also observed features of the human immune landscape that merited an alternative explanation for the accepted pathophysiologic mechanisms of aGVHD (Figure 1). The accepted model requires that host antigen-presenting cells (APCs), which survive pretransplant conditioning, sensitize donor T cells, which in turn attack host tissues sharing the same HLA and miHAs expressed by the host APCs. Prevention of GVHD requires either pharmacologic interference with donor T cell activation or removal of T cells from the allograft. There are several T cell–depletion approaches in use, either by negative selection of T cells or by positive selection of $CD34^+$ progenitors. Some methods, such as targeted depletion using alemtuzumab (anti-CD52), may even remove some APCs in addition to T cells (11). Divito et al. and Jardine et al. have correctly noted, however, that even after nearly complete T cell depletion from allografts, some recipients still develop GVHD. These observations invoked the need for alternative explanations.

Two discoveries from these groups lead in the right direction. In the first, Jardine et al. (10) noted that donor $CD11c^+CD14^+$ monocyte–derived macrophages (12) achieved the greatest fold increase among all leukocytes in GVHD and that these macrophages expressed epitopes indicating recent egress from the circulation (10). The macrophage/DC ratio was also sensitive and specific for GVHD, increasing more than 100-fold in GVHD skin lesions. Compared with steady-state macrophages, these donor monocyte–derived macrophages also secreted inflammatory cytokines and could activate allogeneic T cells. HLA-matched mixed leukocyte reactions (MLRs) confirmed these findings, and the resulting activated macrophages

also mediated cytopathic damage to skin explants (13) independently of T cells.

In a complementary finding by Divito and colleagues, host memory T cells predominated in peripheral tissues and resisted depletion by high-dose conditioning (9). Host T cells also populated GVHD skin lesions and gut, whereas blood contained primarily donor-derived T cells. The majority of APCs in aGVHD were donor derived and frequently observed in contact with host T cells. This T cell abundance in peripheral tissues was further confirmed in a humanized mouse model in which donor monocytes activated cutaneous resident T cells and caused dermatitis akin to GVHD.

Clinical implications and future directions

As Louis Pasteur stated in 1854, "Dans les champs de l'observation le hasard ne favorise que les esprits preparés" (In the fields of observation, chance favors only prepared minds). The complementary findings from these two studies (9, 10) certainly demonstrate the rewards of pursuing inconsistencies in accepted disease mechanisms by their discovery of unique processes underlying what we call GVHD and probably also GVL. These findings go beyond the accepted model of donor T cells from an allograft being sensitized by host APCs, after which donor T cells mediate aGVHD in host target organs. Divito et al. (9) have persuasively demonstrated that host, not donor, T cells, particularly memory T cells, persist in peripheral tissues despite even myeloablative conditioning pretransplant and are present in cutaneous and gut lesions typical of GVHD. Jardine et al. (10) have identified the flip side of this process, which is the predominance of donor instead of host monocyte–derived $CD11c^+CD14^+$ inflammatory macrophages among all myeloid cells in GVHD lesions. These donor macrophages activate allogeneic T cells, which are host not donor, and the same donor macrophages are themselves directly cytopathic. Given that the donor or host origin of the effector T cells determines the vector of graft-host reactivity, this means that what we have always considered to be GVHD includes a component of host-versus-graft (HVG) reactivity.

Other important variables that affect alloHCT outcomes include degree of HLA matching, donor versus recipient sex, myeloablative versus nonablative pretransplant conditioning, bone marrow versus peripheral blood versus umbilical cord blood sources of stem cells, and the specifics of GVHD prophylaxis. Divito et al. detailed these variables (9), but the small patient numbers in these studies preclude direct consideration of these parameters. These variables will be important to include in future analyses.

Neither group has considered the role of another cutaneous myeloid population, epidermal Langerhans cells (LCs). That said, whereas there are mouse models in which GVHD develops in response to LCs (14), other groups have shown these cells are not required (15, 16). LCs are also self-renewing from local progenitors in skin and are replenished by donor-derived populations after alloHCT within approximately 3 months, even after nonmyeloablative conditioning (17). The role of LCs in light of the new information about donor macrophages in skin merits further investigation.

The Divito et al. (9) and Jardine et al. (10) studies do not disprove a role for the canonical model of donor T cell–mediated GVHD after sensitization by host APCs. In fact, these investigators have speculated that higher grades of aGVHD may result from donor T cell reactivity, whereas residual host T cells stimulated by donor macrophages and even the donor macrophages themselves may mediate lower grades of GVHD (9, 10). Another laboratory has independently reported that allogeneic T cell–secreted GM-CSF supports donor phagocyte secretion of inflammatory products that support GVHD, but does not affect allogeneic T cells' capacity to mediate GVL (18). This raises the possibility of uncoupling the ravages of GVHD from the desired benefit of GVL by interfering with T cell–secreted GM-CSF, thus broadening the potential applications of alloHCT and further improving its clinical success.

These studies open new avenues of investigation based on mechanistic questions that remain unresolved. Given these investigators' finding of an active HVG process in what is clinically recognized as GVHD, evaluation of recipients mismatched in the HVG direction, either haploidentical (5) or HLA-DP disparate (19), should prove informative. Prospective T cell chimerism assessment in tissue and blood, as well as determination of whether resident host T cells become anergic or undergo apoptosis during the observed HVG responses, would also be instructive. To ensure well-defined analyses, controlling for degree of HLA matching, intensity of conditioning regimens, allograft source, type of GVHD prophylaxis, and remission status will be essential.

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