

Minor Histocompatibility Antigens-Targets of Graft versus Leukemia Responses

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Abstract

Immune-mediated elimination of tumor cells by donor T cells recognizing recipient minor H antigens contributes to the curative potential of allogeneic HCT. The importance of the allogeneic response to a successful outcome is clearly illustrated by the results of stem cell transplant for malignancy after nonmyeloablative conditioning. Remarkably little is understood about the molecular nature of minor H antigens and this has impeded efforts to determine the role of specific disparities in graft versus tumor reactions or to manipulate T cell responses to augment antitumor activity without exacerbating GVHD. The isolation of minor H antigen-specific CD8⁺ and CD4⁺ T cell clones from recipients of allogeneic HCT has provided the reagents to characterize their expression on leukemic progenitors and to identify the genes encoding these antigens. Using cDNA expression cloning, genetic polymorphisms in the human IFI-75, Uty, KIAA0020, and UGT2B17 genes have been identified to encode new minor H antigens presented by HLA A3, B8, A2, and A29 respectively. Two of these genes are preferentially expressed in hematopoietic cells including leukemic progenitors suggesting it may be possible to augment T cell responses to promote a selective graft versus leukemia effect. A third gene, UGT2B17 is highly expressed in liver and GI tract and may be a target for GVHD in these organs. The studies to identify the molecular nature of minor H antigens have provided insights into the complexities of the graft versus host response associated with allogeneic HCT, but the challenge for the future will be to develop strategies that can selectively induce durable graft versus tumor effects without GVHD. A critical issue in developing specific immunotherapy to augment GVL responses is to determine which minor H antigens are expressed on leukemic stem cells. Studies using transplantation of human AML into SCID mice have identified a putative leukemic stem cell which is contained in the CD34⁺ CD38⁻ subset of the blast population and is present in very low frequency (<1/200,000) in blood or bone marrow from AML patients. We have examined the ability of minor H antigen-specific CTL to prevent engraftment of human AML in NOD/SCID mice. These studies show that engraftment of leukemias derived from individuals encoding the minor H antigen can be specifically prevented demonstrating that AML stem cells express minor H antigens and are targets for CTL. One approach to determine directly which minor H antigens can be selectively targeted to induce a GVL effect without GVHD is to adoptively transfer T cell clones of defined specificity and function to patients who relapse after HCT. Studies of this approach are now in progress in acute leukemia and have provided important insights into potential obstacles of T cell therapy for relapsed leukemia after HCT.

The eradication of leukemia after allogeneic hematopoietic stem cell transplantation (HCT) results both from the cytotoxic chemoradiotherapy administered prior to transplant and immunologic mechanisms mediated by effector cells contained in or derived from the stem cell graft [1]. Two important clinical developments have

evolved from identification of this immune mediated graft-versus-leukemia (GVL) effect. The first is the use of donor lymphocyte infusions (DLI) to treat patients with post transplant leukemic relapse [2-5]. With DLI, the majority of patients with recurrent CML after HCT achieve a complete remission and a smaller but signifi-

cant fraction of patients with other malignancies respond to therapy [3,6]. The second is the development of allogeneic HCT using less toxic nonmyeloablative conditioning regimens [7-12]. With this approach, low doses of irradiation and chemotherapy, which alone are not sufficient to eradicate tumors, are administered to facilitate graft acceptance and tumor regression is induced by donor immune cells. Nonmyeloablative transplants have significant activity for patients with chronic myeloid leukemia, chronic lymphocytic leukemia, myeloma, lymphoma, and renal cell carcinoma [7-12]. The potent antitumor effects observed after DLI and allogeneic HCT in a variety of advanced malignancies represent a remarkable demonstration of the curative potential of immunotherapy and contrasts the difficulty eliciting effective autologous antitumor immune responses to tumor associated antigens by vaccination or cellular therapy [13]. However, with current approaches to allogeneic HCT it has not been possible to separate the beneficial GVL effect from deleterious graft-versus-host disease (GVHD). In this session, I will discuss potential methods for separating GVHD and GVL responses based on our improved understanding of T cell responses to antigens displayed on leukemic cells.

1. Effector Mechanisms in the Graft versus Leukemia Response

A more complete understanding of the cellular mechanisms operative in an allogeneic GVL response should provide critical insights into the requirements for effective antitumor immunity and potentially permit separation of GVL from GVHD. The association of GVL activity with GVHD has implicated donor T cells reacting with minor histocompatibility (H) antigens expressed by recipient cells as major contributors to the GVL effect. The first clinical demonstration of GVL activity was observed after allogeneic HCT for advanced leukemia in which the probability of leukemic relapse was found to be significantly lower in those patients who developed acute and/or chronic GVHD [14,15]. Analysis of patients with CML, AML, or ALL treated with either allogeneic unmodified HCT, allogeneic T cell depleted HCT, or syngeneic HCT showed that the risk of relapse was lowest for patients that received allogeneic unmodified HCT and developed acute and/or chronic GVHD [1,16]. Transplantation with syngeneic or T cell depleted allogeneic marrow to avoid GVHD was associated with a higher risk of relapse unless the conditioning regimen was intensified [1,17,18]. However, GVHD is not a prerequisite for GVL activity. A reduction in relapse was evident in the subset of CML and AML patients that received allogeneic unmodified HCT but did not develop GVHD and remissions have been observed after DLI in the absence of significant GVHD [1,3]. This suggested that there may be antigenic determinants recognized by T cells that would permit the separation of GVL responses from GVHD.

There is evidence that effector mechanisms other than T cells may also contribute to GVL activity either di-

rectly or as a consequence of inflammation induced by allogeneic T cells. NK cells lyse leukemic cells in vitro and may be particularly effective for inducing GVL activity after T cell depleted haploidentical transplant where disparity between killer inhibitory receptors (KIRs) expressed by donor NK cells and HLA molecules on recipient leukemic cells favors NK activation [19,20]. Recently, antibody responses to nonpolymorphic proteins expressed in leukemic cells have been detected in CML patients that have relapsed after an allogeneic HCT and then achieved remission with donor lymphocyte infusion [21]. Thus, while allogeneic T cells are central to the GVL effect after HLA matched HCT and will be the subject of the remainder of this review, other effector mechanisms may contribute to antitumor activity in selected circumstances.

2. Target Antigens for T Cells in Graft-Versus-Leukemia Responses

Clinical and animal studies have identified donor CD8⁺ cytotoxic T cells (CTL) and CD4⁺ helper T cells (Th) as the primary mediators of GVHD and GVL responses after allogeneic HCT between HLA identical individuals [22]. CD8⁺ and CD4⁺ T cells recognize antigenic peptides displayed on the surface of target cells bound to class I or class II MHC molecules, respectively [23,24]. One approach for separating GVL from GVHD is to identify peptides that are recognized by T cells and presented by leukemic cells but not by tissues that are a target of GVHD. There are several broad categories of proteins that may give rise to antigens that could be targets of a selective GVL response. These include a). tumor-specific proteins resulting from chromosome translocations such as bcr/abl or PML/RAR, or from mutations such as p21 ras [25-27]; b). normal proteins that are overexpressed in leukemic cells such as WT-1 or proteinase 3 [28-32]; and c). minor H antigens that are selectively expressed in recipient hematopoietic cells including leukemic cells but not in nonhematopoietic cells [33-37].

The first two categories of antigens are not alloantigens and therefore could be relevant for immunotherapy in the absence of allogeneic HCT. However, it is conceivable that the providing allogeneic cells that have not been previously exposed to the tumor may facilitate eliciting a T cell response to these proteins. Proteins such as p21 ras and bcr/abl are attractive targets for a GVL response because of their tumor-specific expression and involvement in the malignant phenotype. Unfortunately, there is little evidence as yet for activation of donor T cells reactive with such antigens after allogeneic HCT [38]. Studies to elicit bcr/abl-specific responses by vaccination of CML patients with synthetic peptides are in progress [39,40]. The results of these studies should help clarify the immunogenicity of this protein and its relevance as a target for immunotherapy.

Normal proteins that are overexpressed in leukemic cells have been proposed as targets for GVL responses. Proteinase 3 (PR-3) is a serine protease expressed in

normal myeloid differentiation and overexpressed in CML and AML progenitors. CD8⁺ T cell responses to PR-3 were initially demonstrated by stimulating lymphocytes from normal individuals with a peptide that was predicted to bind to HLA A2 [30]. These T cells lysed CML cells and inhibited leukemic colony formation demonstrating that leukemic cells presented this antigen to T cells [30-32]. Moreover, PR-3 reactive T cells had no effect on normal hematopoietic progenitors in vitro [31]. A recent study demonstrated expansion of PR-3 reactive T cells in patients who responded to allogeneic HCT, donor lymphocyte infusion, or interferon supporting the hypothesis that these T cells may participate in a GVL response without affecting normal hematopoiesis or causing GVHD [41]. Similar to PR-3, WT-1 is expressed at high levels in some leukemias but at low levels in normal hematopoietic cells [28,42]. CD8⁺ T cell responses to WT-1 have also been elicited in vitro and inhibit leukemic colony formation without affecting normal colony growth [28,29,43]. Vaccination and T cell therapy trials targeting PR-3 and WT-1 have been proposed. The development of expression array technology to identify genes that are overexpressed in leukemic cells may identify additional antigens in this category or future studies.

The third and broadest class of antigens for a GVL response are minor H antigens. The potency of the GVL effect in allogeneic compared with syngeneic HCT emphasizes the critical importance of minor H antigens for immune mediated eradication of tumors. Minor H antigens are derived from proteins that differ between the donor and recipient due to polymorphism in the genome [44-49]. These polymorphisms may encode changes in amino acid sequence that result in altered binding of the peptides to MHC, contact between the MHC/peptide complex and the T cell receptor, or differential processing of the protein [44-50]. Thus, even though HLA matched siblings express identical MHC molecules on the surface of their cells, the repertoire of peptides displayed in the MHC binding groove may differ substantially due to genetic differences outside the MHC.

Cell culture techniques for characterizing T cell responses to minor H antigens after allogeneic HCT have been developed. Studies analyzing minor H antigen-specific T cell responses have already provided several insights into their potential as targets for GVL therapy. First, minor H antigen-specific T cell clones that lyse recipient hematopoietic cells but not nonhematopoietic cells can be isolated from the majority of HLA identical HCT recipients [34,51]. The observation that expression of some minor H antigens was tissue-restricted was not surprising since hematopoietic and nonhematopoietic tissues express distinct genetic programs that dictate their phenotype and function. However, these results identified a potential strategy for the selective targeting of recipient hematopoietic cells including leukemic cells without causing GVHD. Second, there are a very large number of minor H antigens in the human population. Goulmy et al. have described minor H antigens encoded by autosomes (HA-1 to HA-7) and H-Y antigens encoded

by the Y chromosome genes SMCY and DFFRY [33]. Our group has defined 38 novel minor H antigens recognized by CD8⁺ T cells based on differences in the class I HLA restricting allele, and/or the pattern of recognition of cells from unrelated individuals sharing the HLA restricting allele [34], Warren EH, Riddell SR, unpublished data). Finally, minor H antigens are inherited in a Mendelian fashion [33]. Thus, once a sufficient number of minor H antigens are identified and determined to be involved in GVL responses, tissue typing of donors and recipients can be used to identify appropriate targets for therapy.

3. Identification of Minor H Antigen Genes

The isolation of T cell clones that recognize minor H antigens has provided reagents for characterizing tissue expression of the antigen and for gene identification. The identification of genes encoding minor H antigens recognized by CD8⁺ T cells has progressed more quickly although strategies for antigen identification for CD4⁺ T cells have recently been developed [52]. Three methods are being applied to the discovery of genes encoding human minor H antigens recognized by CD8⁺ T cells. These include peptide elution and mass spectrometry [53], cDNA expression cloning [54], and genetic linkage analysis [55]. Goulmy and Engelhard have eluted peptides from class I MHC molecules, separated fractions that reconstitute T cell recognition, and sequenced the active peptides by mass spectrometry [44, 45,47,48,56]. Their studies have identified the amino acid sequence for five minor H antigens. A search of DNA and protein databases revealed that Y chromosome genes SMCY and DFFRY encode three of the minor H antigens. SMCY and DFFRY are broadly expressed in both hematopoietic and nonhematopoietic tissues suggesting that T cell responses to these antigens may mediate GVHD in addition to GVL activity [48,56]. Indeed, the development of T cell responses to the SMCY/HLA A2 minor H antigen was associated with the development of acute GVHD after HCT from a female donor to a male recipient [57].

An HLA A2-restricted minor H antigen termed HA-1, was found to be encoded by an autosomal gene (KIAA0223), and another, termed HA-2, had homology to a sequence in the class II myosin gene [44,45]. Both of these minor H antigens are selectively expressed in hematopoietic cells and have been proposed as targets for a GVL response. HA-1 incompatibility was associated with a lower rate of leukemic relapse in one small study but other studies have linked HA-1 incompatibility with GVHD [57-59]. This was surprising based on its tissue expression. However, HA-1 is highly expressed in dendritic cells (DC), which have a critical role in the induction of GVHD [60]. Thus, it is conceivable that local inflammation initiated by T cells responding to HA-1 expressed by recipient DC in tissues might lead to recruitment of T cells responding to other minor H antigens expressed on epithelial cells. Additional studies are needed to resolve the role of this

minor H antigen in GVHD and GVL responses.

In collaboration with the Engelhard lab, we have also used peptide elution to discover an HLA A2-restricted minor H antigen termed HA-8, which is encoded by the KIAA0020 gene [50]. KIAA0020 is broadly expressed in both hematopoietic and nonhematopoietic cells. Preliminary studies examining 577 HLA A2⁺ allogeneic HCT recipients demonstrated that recipients who express HA-8 and have HA-8 negative donors have an increased risk of both acute and chronic GVHD [61], Akatsuka Y, unpublished data). The broad tissue distribution of KIAA0020 and the clinical association of HA-8 incompatibility with GVHD suggest HA-8 would not be suitable as a GVL target.

Our lab has also used the cDNA expression cloning methodology pioneered by Boon et al. [54], to identify four novel genes encoding minor H antigens. One of the genes identified is the Y chromosome gene UTY that encodes a peptide presented by HLA B8 [49]. In contrast to CTL specific for SMCY which are associated with GVHD, the CTL specific for UTY were isolated from an allogeneic HCT recipient without GVHD and lysed hematopoietic cells including leukemic blasts but not skin fibroblasts. By RNA analysis, UTY is highly expressed in hematopoietic cells and is low in most nonhematopoietic tissues. Thus, we have searched for additional epitopes in UTY that may be presented by other common HLA alleles. There are three transcripts of UTY that encode proteins of 1079, 1240, and 1347 amino acids, respectively [62]. The coding sequence of all three UTY transcripts is identical over the first 1079 amino acids and transcripts 2 and 3 are identical over 1240 amino acids. Comparison of UTY with the UTX homologue encoded by the X chromosome showed only 80 to 84% homology suggesting there will be epitopes presented by other HLA alleles. This was validated by the recent discovery of a UTY peptide presented by HLA B60 [63]. Thus, UTY may be broadly applicable for a GVL response in male recipients of HCT from female donors. The genes encoding three additional minor H antigens that are encoded by autosomes and selectively presented to CTL by hematopoietic cells and leukemic blasts have recently been identified with this approach.

A third approach for identifying minor H antigen genes involves genetic linkage analysis using Epstein Barr virus transformed B cell lines established from the Centre d'Etude Polymorphisme Humain (CEPH) reference families that have been extensively mapped for genetic markers. Completion of the Human Genome Project should increase the probability that this approach will identify candidate genes rather than simply provide a chromosomal location of the minor H antigen and it is anticipated that it will be more extensively used in the future [55].

4. Expression of Minor H Antigens on Leukemic Cells

An essential criteria for selection of minor H antigens

to induce a GVL response is expression of the antigen on leukemic cells. The sensitivity of leukemic cells to minor H antigen-specific T cells has relied on assays of the ability to T cells to lyse radiolabeled leukemic blasts, or to inhibit leukemic colony formation in soft agar [34,64,65]. However, it is unclear if these assays detect recognition of leukemic stem cells. Transplantation of human leukemia into NOD/SCID mice has identified a putative stem cell, which is essential for establishing leukemic hematopoiesis [66,67]. The NOD/SCID model has now been adapted for studies to assess recognition of this SCID leukemic initiating cell (SL-IC) by minor H antigen-specific CTL [68]. Studies in our lab examining CD8⁺ CTL specific for 5 distinct minor H antigens including the antigen encoded by UTY demonstrate that SL-IC could be specifically eliminated by minor H antigen-specific CTL [68].

5. Strategies for Exploiting the Graft versus Leukemia Effect

5.1. Donor Lymphocyte Infusions

Relapse of the malignancy remains a frequent cause of treatment failure for patients who undergo allogeneic HCT. The recognition that donor T cells mediate a GVL effect led to efforts to augment this effect in patients with advanced disease by infusing additional donor lymphocytes early post transplant. This was complicated by a high incidence of severe GVHD and an increase in non-relapse mortality [69]. However, donor lymphocyte infusions given later after HCT to patients with relapsed leukemia have induced GVL effects with more modest toxicity. Approximately 75% of patients with recurrence of CML in the chronic phase and up to 25% of patients with AML or ALL have achieved a complete remission after DLI [2-5]. GVHD remains a major complication of DLI occurring in 50 to 65% of patients and contributes to an 18% probability of death in remission at one year [3]. Efforts to control GVHD while preserving antileukemic activity of DLI using graded doses of lymphocytes or depletion of the CD8⁺ T cell subset have been partially effective but have not permitted the complete separation of GVL from GVHD [70,71]. The results of DLI validate the concept that the GVL effect can be augmented by immunotherapy but the development of GVHD in the majority of treated patients and the low response rate in patients with acute leukemia remain significant limitations.

5.2. Suicide Genes

Methods have been developed to introduce genes into T cells and confer novel functions. The HSV thymidine kinase (HSV-TK) gene has been evaluated as an inducible suicide gene to regulate the survival of donor T cells and ameliorate GVHD. Unlike the mammalian TK, HSV-TK efficiently phosphorylates ganciclovir leading to the formation of toxic di and triphosphate moieties that

interfere with DNA replication in dividing cells. HSV-TK was introduced into T cells in adoptive transfer studies of virus-specific CTL in nontransplant patients. In this setting, the transduced T cells were eliminated by an immune response to the foreign TK transgene product [72]. A similar outcome was observed in HCT recipients of unmodified stem cell grafts that received TK-modified DLI to treat leukemic relapse more than one year after transplant (Flowers, M.E.D., Riddell S.R. unpublished data). However, Bordignon et al. used HSV-TK modified DLI to treat relapsed leukemia or EBV lymphoproliferative disease in recipients of T cell depleted allogeneic HCT and observed immune responses to the transgene product in only a minor subset of patients [73]. Moreover, their study demonstrated the successful ablation of transduced T cells and reversal of GVHD with ganciclovir administration.

Alternative transgenes for inducing cell suicide that may be less immunogenic are in development. One approach based on the expression of Fas that naturally signals apoptosis in T lymphocytes, uses a fusion gene that encodes 3 human proteins [74]. These include the extracellular and transmembrane domains of the human low affinity nerve growth factor receptor (LNGFR) to provide a marker for selection, two copies of human FKBP12 containing a single point mutation to provide high affinity binding to a drug AP1903, and the intracellular domain of Fas to signal cell death. This fusion gene is inert until addition of AP1903, which binds AP1903, clusters the chimeric Fas molecules, and induces apoptosis [74]. This approach does not require cell division for the induction of cell death and the human origin of the transgene product should reduce the probability it will be immunogenic.

5.3. Antigen-Specific T Cell Clones

The use of antigen specific T cell clones rather than polyclonal donor lymphocytes might provide a more potent antileukemic effect and reduce the risk of GVHD. CMV and EBV-specific T cells have been isolated from allogeneic donors, cultured in vitro and adoptively transferred to the respective recipient without causing GVHD. Moreover, these studies have demonstrated that transferred T cells can persist in vivo, migrate to sites of antigen, and exert effector function [75-77]. The identification of minor H antigens and leukemia associated proteins such as WT-1 and PR-3 suggests a similar approach may be used to augment the GVL effect without causing GVHD. Initial studies to assess the adoptive transfer of T cell clones to patients with post transplant leukemic relapse are already in progress and should provide insight into the safety of this approach.

Studies in animal models and in humans have identified obstacles that may limit the therapeutic success of T cell transfer. The elimination of leukemia may require prolonged persistence of the transferred T cells suggesting that CD4⁺ Th cells or IL-2 may be required [78]. Tumors may evade T cell recognition by loss of

antigen or MHC expression [79], or inducing functional anergy in tumor-reactive T cells [80]. Thus, to provide insights into limitations of antigen-specific T cell therapy for leukemia, it will be essential to monitor persistence, migration, and function of transferred T cells, and to examine alterations in tumor cells if relapse occurs after therapy. This information can then be used to guide subsequent studies and hopefully lead to the successful incorporation of specific T cell therapy into allogeneic HCT regimens.

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