William Wolk. *Alonzo.* Oil on canvas, 24″ × 30″.

Results of preclinical studies suggest continued efforts in T-cell therapy will establish immunotherapy as a useful modality in clinical practice.

T-Cell Therapy of Leukemia

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Background: *The demonstration that immune-mediated elimination of leukemia contributes to the success of allogeneic hematopoietic stem cell transplantation (HSCT) has renewed interest in the development of immune-based therapies that might be used to augment the antileukemic effect of HSCT or in patients who are not receiving HSCT.*

Methods: *The authors reviewed studies that have analyzed the mechanisms that may be operative in T-cell recognition of leukemia after allogeneic HSCT, identified candidate target antigens for immunotherapy of leukemia in transplant and nontransplant patients, and evaluated expression of candidate antigens on leukemic progenitors.*

Results: *A large number of potential targets for T-cell therapy or vaccination have now been identified in human leukemia. Studies to evaluate novel immune-based therapies are now being initiated.*

Conclusions: *The rapid pace of progress in cellular and molecular immunology has identified new opportunities for developing T-cell therapy or vaccination for leukemia. Obstacles must be addressed before these approaches can be applied broadly, but the promising results of preclinical studies suggest continued efforts in this area will result in the establishment of immunotherapy as a useful modality in clinical practice.*

Introduction

Experiments in animal models have demonstrated the potential of immune-based approaches for cancer therapy. Antibody and cytokine therapy have already been successfully developed and incorporated into standard treatment regimens for some human malignancies. The development of cellular immunotherapy with effector cells of defined specificity and function has proven more challenging, but the rationale for pur-

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suing this approach is supported by the results in animal models as well as by clinical observations.

In murine models, the adoptive transfer of CD8+ or CD4+ T cells specific for tumor associated or minor histocompatibility antigens (mHAgs) expressed by leukemic cells provides a potent antileukemic effect and converts the incomplete responses achieved with chemotherapy into cure.[1-3] There is compelling evidence, much of which is derived from the results of allogeneic hematopoietic stem cell transplantation (HSCT), that human leukemias can also be recognized and eliminated by T cells. The immunologically mediated graft-vs-leukemia (GVL) effect that was predicted by animal model studies of allogeneic HSCT has been documented in clinical trials. Patients who receive an allogeneic transplant for advanced leukemia have a lower probability of leukemic relapse if they develop acute and/or chronic graft-vs-host disease (GVHD) as a complication of the transplant.[4,5] The risk of leukemic relapse is increased after syngeneic HSCT or T-cell-depleted allogeneic HSCT, suggesting a critical role for donor T cells specific for allogeneic determinants in initiating or mediating the GVL effect. Although the GVL effect is most prominent in patients with GVHD, a reduction in relapse is also evident in patients without GVHD, thus demonstrating that clinical GVHD is not a prerequisite for GVL activity.[6] The type of leukemia is also a factor in the GVL effect associated with allogeneic HSCT. The reduction in relapse attributed to donor T cells is greatest for chronic myeloid leukemia (CML), intermediate for acute myeloid leukemia (AML), and lowest for acute lymphoblastic leukemia (ALL).[6] The importance of the GVL effect to a successful outcome after allogeneic HSCT is well established, but relapse and GVHD remain significant obstacles, especially for patients with advanced acute leukemia. Approaches to augment the GVL effect without GVHD would be a welcome therapeutic development.

Donor Lymphocyte Infusions After Allogeneic HSCT

The first efforts to augment the GVL effect of allogeneic HSCT to prevent or treat relapse of leukemia did not attempt to separate GVHD from the GVL effect and revealed the difficulties in manipulating immunologic responses with an incomplete understanding of the mechanisms and target antigens involved. The observation that donor T cells were required for the GVL effect suggested that simply infusing additional T cells after transplant might achieve a stronger antileukemic effect. Thus, donor T lymphocytes that were not selected for preferential recognition of recipient leukemic cells were administered in the first 11 days after unmodified HSCT to patients at high risk for posttransplant relapse. Unfortunately, this approach resulted in a dramatic increase in severe acute GVHD and in early mortality.[7] However, the administration of donor lymphocytes later after allogeneic HSCT, when the inflammation and tissue injury caused by the conditioning regimen had subsided, provided more encouraging results, particularly for patients with CML. A durable complete remission was achieved in 50% to 80% of patients who had relapsed with chronic-phase CML after allogeneic HSCT and then received immunotherapy with unselected donor lymphocytes.[8-10] GVHD was less severe than that observed with the early administration of donor T cells but still occurred in 50%-60% of patients and contributed to mortality in approximately 20% of patients.[10] Several approaches have been developed to reduce GVHD after donor lymphotype infusion (DLI) including the infusion of graded doses of T cells, the introduction of inducible suicide genes into transferred T cells, and the administration of selected T-cell subsets. In small studies, these approaches reduced the incidence and/or severity of GVHD and may be beneficial for patients with CML.[11-13]

DLIs have been less effective for patients with relapse of AML, with response rates of <20%. They are rarely successful for patients with relapse of ALL after allogeneic HSCT.[10] It is unclear if the reduced efficacy of DLIs for AML and ALL is related to a lack of antigen expression on tumor cells, alterations in adhesion or other molecules that contribute to T-cell recognition, or the difficulty overcoming an expanding tumor burden with immunotherapy.

The results with unselected DLIs clearly showed that immunotherapy could cure leukemia but have also identified challenges for future research. First, GVHD remains a major obstacle, and strategies that would augment the beneficial GVL effect without causing GVHD are needed. Second, the therapy is less effective for acute leukemia, and novel strategies to induce a more potent antileukemic effect are required for these patients. Third, cellular therapy with donor lymphocytes is applicable only to patients who receive an allogeneic HSCT. Immunotherapy based on isolation of autologous T cells that recognize leukemia would have broader application.

Effector Mechanisms in the GVL Response

The observations that leukemic cells can be eradicated by immunologic responses elicited after allografting or DLIs has led to efforts to identify the effector mechanisms that are responsible. Natural killer

Classes of Target Antigens for T-cell Therapy of Leukemia

Minor histocompatibility antigens in allogeneic HSCT recipients Examples: HA-1, HA-2, HB-1, UTY
Leukemia-specific proteins Examples: bcr/abl, PML/RARα, EMV/AML-1
Leukemia-associated proteins Examples: proteinase-3, WT-1, h-TERT, hdm-2

(NK) cells, which are regulated by the interplay of activating and inhibitory receptor/ligand interactions,[14] as well as antigen-specific T cells have antitumor activity against leukemia in animal models. Clinical studies of recipients of T-cell–depleted haploidentical HSCT have suggested NK cells provide an important contribution to eradicating leukemia. In donor/recipient pairs where there is a mismatch between the killer inhibitory receptors expressed by donor NK cells and the class I major histocompatibility complex (MHC) ligands expressed by the recipient, the relapse rate is markedly reduced.[15] It is hoped that as the receptor ligand interactions involved in regulating NK cells are further elucidated, opportunities for using NK cells or NK cell subsets for leukemia therapy after HLA-identical HSCT or in the nontransplant setting will be identified. However, most of the investigation into cellular immunotherapy of leukemia has been concentrated on identifying T-cell responses to candidate proteins expressed in leukemic cells. Thus, the remainder of this review will focus on current efforts to identify antigen-specific T cells that might have utility for leukemia therapy after allogeneic HSCT and in the nontransplant setting.

Two subsets of mature T cells express the αβ T-cell receptor. CD3+ CD8+ cytotoxic T cells recognize short peptides of 8–11 amino acids derived from intracellular proteins and displayed on the surface of cells associated with class I MHC molecules. CD3+ CD4+ helper T cells recognize peptides derived from intracellular proteins or proteins that have been taken up by endocytosis and presented at the cell surface by class II MHC molecules. Both subsets of T cells have antileukemic activity in animal models. However, because of the ability of CD8+ T cells to directly lyse their target cells, much of the effort in identifying leukemia-reactive T cells has focused on this subset. Several classes of proteins expressed by leukemic cells have been identified to provide peptide epitopes that are recognized by CD8+ or CD4+ T cells. These include mHAgs that are relevant as targets after allogeneic HSCT, and leukemia-specific or leukemia-associated proteins that may be targets in both transplant and nontransplant settings (Table).

mHAgs: Targets for GVL Responses After Allogeneic HSCT

The potency of the GVL effect in allogeneic HLA-identical HSCT recipients compared with syngeneic recipients emphasizes the critical importance of disparity at mHAg loci for immune-mediated eradication of leukemia. mHAgs are peptides that are recognized by donor T cells and are derived from proteins that differ between the donor and recipient due to polymorphism in the genome. The polymorphisms that give rise to mHAgs may affect gene expression or 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.[16] Thus, even though HLA-matched siblings express identical MHC molecules on the surface of their cells, the repertoire of peptides displayed in the peptide-binding groove of these molecules may differ substantially due to genetic differences outside the MHC.

Isolation of mHAg-Specific T Cells

T-cell responses to mHAgs are responsible for both GVL activity and GVHD. Thus, a critical issue is how targeting mHAgs might be used to separate GVL from GVHD. One possibility, which is supported by in vitro data, is to augment T-cell responses specific for mHAgs that are expressed on hematopoietic cells, including leukemic cells, but have limited or absent expression on nonhematopoietic cells that are recognized in GVHD (Fig 1). Cell culture techniques for isolating and characterizing donor T-cell responses to recipient mHAgs after allogeneic HSCT have been developed and have assisted in defining the tissue expression of mHAgs and providing the reagents for gene identification. These methods usually rely on stimulating donor T cells obtained from the recipient after HSCT with gamma-irradiated pretransplant recipient peripheral blood mononuclear cells as antigen presenting cells. With this approach, mHAg-specific CD8+ T-cell clones can be isolated from the majority of HLA-identical HSCT recipients, and a reasonable fraction of the T-cell clones have been found to lyse recipient hematopoietic cells but not nonhematopoietic cells.[17,18] The observation that the expression of some mHAgs was tissue-restricted was not surprising since hematopoietic and nonhematopoietic tissues express distinct genetic programs, and support the feasibility of targeting mHAgs to augment GVL responses without exacerbating GVHD.

Several groups are now actively engaged in isolating and characterizing T cells specific for human mHAgs from allogeneic HSCT recipients. The results of published studies suggest that there will be a large

number of mHAgs in the human population. Goulmy et al[19] have described CD8+ T-cell clones specific for seven mHAgs encoded by autosomes (HA-1 to HA-7) and three H-Y antigens encoded by Y chromosome genes. Our group has defined 38 novel mHAgs recognized by CD8+ T cells based on differences in the class I HLA-restricting allele or the pattern of recognition of cells from unrelated individuals sharing the HLA-restricting allele.[18] This likely represents only a small fraction of the overall number of mHAgs in the human population that could be targets for GVL and GVHD after allogeneic HSCT. For leukemia therapy, however, it is not essential that all mHAgs be identified. The polymorphic genes that encode mHAgs are distributed in the population, and the identification of a relatively small number of mHAgs that are presented by common HLA alleles and are involved in GVL responses could provide therapeutic opportunities in a majority of patients.

Identification of Genes That Encode Minor H Antigens

The identification of the genes that encode mHAgs is helpful for defining the tissue expression of the antigen and essential to elucidate the molecular basis for antigenicity. There are established methods for identifying genes encoding mHAgs recognized by CD8+ T cells, and strategies for identifying mHAgs recognized by CD4+ T cells have recently been developed.[20-23] Three methods are being applied to the discovery of genes encoding human mHAgs recognized by CD8+ T cells. These include peptide elution and mass spectrometry,[20,24-27] cDNA expression cloning,[28,29] and genetic linkage analysis.[22] Investigators have eluted peptides from class I MHC molecules, separated fractions that reconstitute T-cell recognition, and sequenced the active peptides by mass spectrometry.[24-27] Their studies have identified the amino acid sequence for five mHAgs.[19,24-27] A search of DNA and protein databases revealed that Y chromosome genes SCMCY and DFFRY encoded three of these mHAgs. 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. In a subsequent study, the development of T-cell responses to the SMCY peptide presented by HLA-A2 was associated with the development of acute GVHD after HSCT from female donors to male recipients.

An HLA-A2–restricted mHAg 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.[24,25] Both of these mHAgs are selectively expressed in hematopoietic cells and have been proposed as targets for a GVL response without GVHD. Genotyping of HSCT donors and recipients at the HA-1 locus has been performed in an effort to determine if incompatibility at HA-1 influences the outcome of HSCT. HA-1 incompatibility was associated with a lower rate of leukemic relapse in one small study supporting its potential use as a target to augment the GVL effect, but other studies have linked HA-1 incompatibility with GVHD.[30,31] This was not expected since HA-1 is expressed only in recipient hematopoietic cells. However, HA-1 is highly expressed in dendritic cells, which have been shown in murine models to be critical for the induction of GVHD.[32] Thus, it is conceivable that local inflammation initiated by T cells responding to HA-1 expressed by recipient dendritic cells in tissues might lead to recruitment of T cells responding to other mHAgs expressed on epithelial cells, analogous to the epitope spreading that is observed in autoimmune diseases.

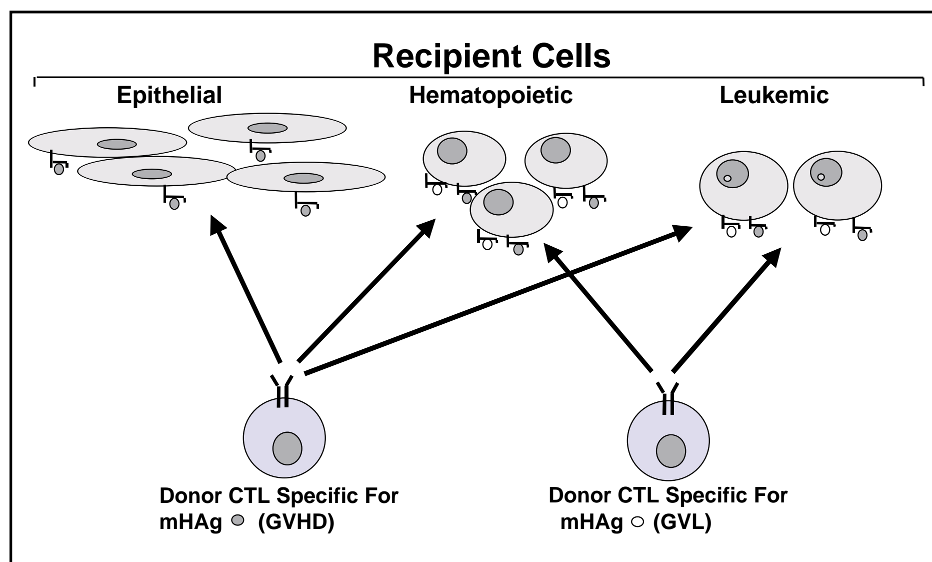

Fig 1. — Tissue-specific recognition of mHAgs may permit separation of GVL and GVHD. Donor T cells specific for mHAgs that are broadly expressed by both hematopoietic and nonhematopoietic cells have been implicated in GVHD. Donor T cells specific for mHAgs that are expressed preferentially or selectively in recipient hematopoietic cells including leukemia progenitors may be targets for a GVL response without GVHD.

In collaboration with investigators at the University of Virginia, 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.[33] KIAA0020

is broadly expressed in both hematopoietic and nonhematopoietic cells and the CD8+ T-cell clone specific for HA-8 was isolated from a patient with GVHD. The critical polymorphism in the HA-8 epitope is a proline (^{P}) to arginine (^{R}) substitution at position 1 of the peptide, which facilitates transport of the HA-8 peptide into the endoplasmic reticulum where it binds HLA-A2. Preliminary studies examining 577 HLA-A2+ allogeneic hematopoietic cell transplant (HCT) recipients demonstrated that those who express the HA-8^{R} allele and have HA-8^{P} donors have an increased risk of acute GVHD (Y. Akatsuka, S. R. Riddell, unpublished data, 2002). 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.

Screening of cDNA libraries prepared from mHAg-positive cells has also been used to identify genes encoding minor H antigens. Dolstra and colleagues[29] identified a gene, HB-1, that encodes an mHAg presented by HLA-B44 and expressed only in transformed B cells including B-cell ALL. The polymorphism in HB-1 is a single amino acid change at position 8 of the HB-1 peptide. The expression of HB-1 on B-cell ALL but not in nonhematopoietic cells suggests it may provide a target for immunotherapy after allogeneic HSCT for ALL in HLA-B44 positive recipients.

It has previously been shown that male recipients of allogeneic HSCT from female donors have a lower risk of leukemia relapse than other donor/recipient gender combinations. One of the mHAg genes we have identified by cDNA transfection is the Y chromosome gene UTY that encodes a peptide presented by HLA-B8.[28] The B8/UTY peptide differs from the UTX homologue expressed in female cells by two amino acids. CD8+ T cells specific for UTY were isolated from a male who received an allogeneic HCT from a female donor and did not develop significant GVHD. B8/UTY-specific cytotoxic T lymphocyte (CTL) lysed hematopoietic cells including leukemic blasts but did not lyse nonhematopoietic target cells in vitro. RNA analysis showed that UTY is expressed at high levels in hematopoietic cells and at lower, but detectable, levels in most nonhematopoietic tissues.[28] UTY has additional polymorphisms with UTX and is likely to encode peptides that bind to other HLA alleles. Thus, studies are in progress to determine if UTY might serve as a general target for a GVL response in male recipients of HSCT from female donors.

A third approach for identifying minor H antigen genes involves genetic linkage analysis using Epstein Barr virus transformed B cell lines. These cell lines were established from the Centre d'Etude Polymorphism 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.[22]

Expression of Minor H Antigens on Leukemic Cells

An essential criterion 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 mHAg-specific T cells was initially assessed by the ability of T cells to lyse Cr^{51}-labeled leukemic blasts or to inhibit leukemic colony formation in soft agar.[18,19] However, the leukemic cell population is composed of a hierarchy of cells at distinct stages of differentiation, and most leukemic cells have a limited capacity for self-renewal. Thus, it cannot be assumed based on the results of CFU inhibition and Cr^{51} release assays that all leukemic cells express the mHAg and are sensitive to T-cell recognition. A putative leukemic stem cell has

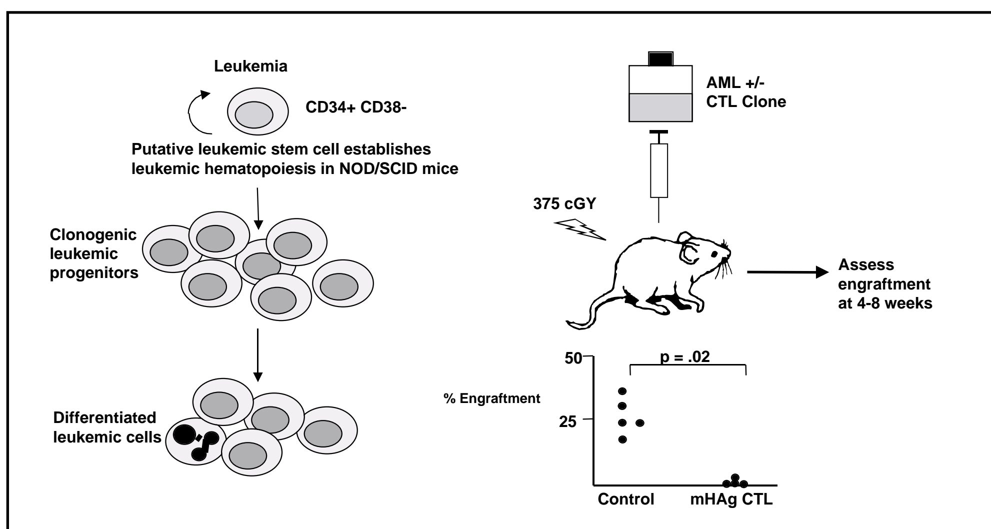

Fig 2. — Evaluating T-cell recognition of leukemia progenitors using NOD/SCID mice. The leukemia cell population consists of a hierarchy of cells with distinct capacity for self-renewal. A putative stem cell is required for engraftment in irradiated NOD/SCID mice. The ability to purge the leukemia population of cells capable of establishing leukemic hematopoiesis by co-culture with antigen-specific T cells can be used to assess recognition of the leukemic stem cell.

been described for AML and ALL based on analysis of the phenotypic subsets required for engraftment after transplantation of human leukemia into immunodeficient mice.[34,35] The immunodeficient mouse model (nonobese diabetic/severe combined immunodificient [NOD/SCID]) of human leukemic hematopoiesis has been adapted for studies to assess recognition of the leukemic stem cell by mHAg-specific CTL (Fig 2). CD8+ CTL specific for five distinct minor H antigens including the antigen encoded by UTY could specifically eliminate leukemic engraftment in this model.[36] As additional mHAgs are identified and considered as potential targets for immunotherapy to induce GVL activity, the NOD/SCID model may have utility in selecting those which are most appropriate to evaluate in a clinical setting.

Leukemia-Associated Proteins: Targets for Immunotherapy in HSCT and Nontransplant Patients

Categories of proteins other than mHAgs are expressed in leukemic cells and are being evaluated as potential targets for immunotherapy in settings that would not require allogeneic HSCT.

Leukemia-Specific Proteins

Leukemia-specific proteins that are expressed as a consequence of chromosome translocations or mutations in cellular genes represent one category of candidate antigens for T-cell immunotherapy. Examples of this class of proteins include the bcr/abl fusion protein resulting from the t9,22 translocation in CML, the PML/RARα fusion protein resulting from the t15,17 translocation in acute promyelocytic leukemia, and the ETV6-AML1 fusion protein in childhood ALL.[37-39] These proteins are attractive for immunotherapeutic approaches because they exhibit selective expression on tumor cells, which limits the potential for toxicity to normal tissues, and may contribute to the malignant phenotype, which makes it less likely the tumor can evade immune recognition by loss of antigen expression. However, there are limitations of fusion proteins as target antigens. The fusion sites may give rise only to peptides that bind strongly to a few MHC molecules. Moreover, even if peptides derived from sequences surrounding the fusion site are identified that bind to MHC, it is essential that these peptides are generated by proteosomal cleavage, bind to the MHC molecules in the ER, and be displayed at the surface of leukemic cells for T-cell recognition.

Studies of the bcr/abl fusion site are the most advanced and have provided provocative data. CD4+ T cells specific for bcr/abl fusion peptides presented by a variety of class II MHC alleles including DR4, DRB1*0901, and DRB5*0101 have been described.[39,40] Peptides spanning the bcr/abl fusion junction have been identified that bind to the HLA-A3, -A11, and -B8 class I molecules.[37] These bcr/abl peptides have been used in vitro to elicit reactive T cells that recognize peptide-pulsed target cells. What has been less clear until recently is whether CML cells actually present bcr/abl peptides at the cell surface. This issue has now been partially addressed. Peptide mixtures eluted from HLA-A3 molecules at the surface of primary CML cells were analyzed by mass spectrometry, and a peptide derived from the bcr/abl junction was identified, providing direct evidence that leukemic cells can process and present bcr/abl derived peptides to CD8+ T cells.[41] These data provide a rationale for attempting to establish bcr/abl reactive T-cell responses in vivo in CML patients either by vaccination or by adoptive cell therapy.[42]

Leukemia-Associated Normal Proteins

A second category of proteins considered to be potential targets for immunotherapy are nonmutated proteins that are overexpressed or preferentially expressed in leukemic cells compared with normal cells. The rationale for investigating such proteins as targets for leukemia-specific T-cell therapy comes largely from studies of solid tumors. In melanoma, normal proteins including tyrosinase, gp100, gp75, and MART1, which are involved in melanocyte differentiation, and cancer-testes antigens including the MAGE proteins, which have limited expression in normal tissues, have been identified as targets for tumor-specific T cells. Similarly, in leukemic cells, normal leukemia-associated proteins that are not mutated have been shown to contain epitopes recognized by CD8+ T cells. A few examples of such proteins in leukemia include proteinase-3, wt-1, hdm2, and human telomerase reverse transcriptase (hTERT). In most cases, these proteins have also been suggested to contribute to the malignant phenotype.

Proteinase-3, a primary granule enzyme that is predominantly expressed in normal promyelocytes but is overexpressed in myeloid leukemia cells, was the first leukemia-associated protein to be studied in detail as a target for T cells. The sequence of proteinase-3 was scanned using MHC peptide-binding algorithms to identify peptides that were predicted to bind to the HLA-A2 class I MHC molecule.[43] A peptide termed PR-1 was shown to bind to HLA-A2 with high affinity and was used to pulse antigen-presenting cells and stimulate T cells from HLA-A2+ individuals. CD8+ T cells were isolated that killed PR-1 peptide-pulsed autolo-

gous target cells and HLA-A2+ CML and AML cells. These data indicated that CD8+ T cells reactive with the PR-1 self-peptide could be elicited from the T-cell repertoire of HLA-A2+ individuals. These T cells inhibited the production of leukemic colony-forming units/granulocyte-macrophage (CFU-GM) colonies but not normal CFU-GM colonies, suggesting recognition was selective for leukemic cells perhaps because of higher expression levels of the protein in tumor cells.[43] Recent studies using an HLA-A2/PR-1 peptide tetramer to directly stain and quantitate PR-1– reactive T cells in vivo has demonstrated that functional PR-1–reactive T cells are expanded in CML patients who have responded to interferon α or allogeneic HSCT.[44] This data does not provide conclusive evidence for antileukemic activity of PR-1–reactive T cell, but is important for demonstrating that the presence of large numbers of PR-1–specific T cells in vivo does not interfere with normal hematopoiesis. These results have led to the development and evaluation of peptide-based vaccines to elicit PR-1–specific T-cell responses in leukemia patients and are likely to lead to trials of adoptive immunotherapy with PR-1–specific T cells.

WT-1 is a zinc finger transcription factor that was initially thought to be a tumor suppressor based on studies in Wilms' tumor. However, subsequent studies showed that WT-1 was overexpressed in many malignancies, and it has been implicated in maintaining the malignant phenotype. WT-1 is expressed in normal cells in the kidney, testes, ovary, uterus, and lung, and it is expressed at low levels in normal CD34+ hematopoietic cells.[45] High levels of expression of WT-1 are observed in AML, ALL, and CML, and it has been used as a molecular marker to detect relapse of leukemia. Recent studies have suggested WT-1 may be a suitable target for cellular immunotherapy of leukemia. The sequence of WT-1 was scanned for peptides that bind to class I molecules and peptides that bind to HLA-A2 and -A24 were identified.[46,47] These peptides have been used to elicit T cells reactive with WT-1 in vitro. WT-1 specific T cells have antileukemic activity in vitro and eliminate leukemic progenitors in immunodeficient mice engrafted with human leukemia.[48]

Telomerase is a ribonucleoprotein enzyme that is required to maintain telomere length and plays a role in cellular replicative life-span. Human telomerase reverse transcriptase (hTERT) is one component of the complex and is highly expressed in most tumor cells including leukemia. Peptides in hTERT that bind to HLA-A2 and -A24 were used to pulse antigen-presenting cells and isolate T cell lines and clones that recognize tumor cells expressing high levels of endogenous hTERT.[49,50] Preliminary studies suggest that hTERT-specific CTLs do not recognize normal hematopoietic cells in vitro, although the more rigorous evaluation of effects on engraftment in NOD/SCID mice have not been performed.[49]

The human homologue of the *mdm-2* oncoprotein, hdm-2, is another self-protein in the category of leukemia-associated proteins that are involved in malignant transformation. Hdm-2 is overexpressed in a variety of malignancies and inactivates the p53 tumor suppressor protein. In contrast to proteinase-3, WT-1, and h-TERT, the use of peptides derived from hdm-2 and predicted to bind to class I has not been successful in eliciting hdm-2 reactive T cells, suggesting that tolerance to this normal protein is more complete.[51] However, high-avidity T cells specific for hdm-2 can be elicited by immunizing HLA-A2 transgenic mice with hdm-2 peptides or by stimulating T cells from HLA-A2–donors with HLA-A2+ cells pulsed with hdm-2 peptide. The T-cell–receptor α and β genes were cloned from such high-avidity T cells and introduced into normal T cells from HLA-A2+ donors to engineer T cells that are reactive with hdm-2+ tumor cells for potential use in adoptive immunotherapy.[51]

Strategies for Inducing a T-Cell–Mediated Antileukemic Effect

Adoptive Therapy With Antigen-Specific T-Cell Clones

The adoptive transfer of donor T cells specific for antigens expressed by cytomegalovirus (CMV) or Epstein Barr virus (EBV) has been shown to restore CMV- and EBV-specific immunity after allogeneic HSCT without causing GVHD.[52,53] These studies have demonstrated that it is feasible to isolate T cells of desired specificity, and expand cells in vitro that retain function and the ability to persist and migrate in vivo after infusion to patients. The adoptive transfer of T cells selected for specificity for mHAgs expressed by recipient leukemic cells represents a potential approach for eradicating leukemic cells without GVHD in the allogeneic HSCT setting and for inducing a more potent antitumor effect than achieved with unselected polyclonal DLIs.

The process of isolating and expanding mHAg-specific T cells for use in therapy is still challenging but will improve as a larger number of mHAgs are characterized at the molecular level. A potential problem with targeting mHAgs in the allogeneic setting is GVHD. Similarly, toxicity to normal tissues could occur as a result of transferring T cells specific for self-proteins such as proteinase-3, WT-1, hTERT, or hdm2 in the nontransplant setting. One approach for defining safe-

ty is to introduce a suicide gene into the T cells that could be activated if toxicity occurred. The herpes virus thymidine kinase (TK) gene has been used in clinical studies and has been shown to be effective in reversing GVHD after DLI.[13] However, TK is immunogenic and can result in the premature elimination of transferred T cells that do not cause toxicity.[54] A suicide gene based on inducing cell death through the Fas pathway using chemical dimerizers to activate an engineered transgene based entirely on human proteins has been developed and may circumvent the problem of immunogenicity.[54]

Vaccination

An alternative or potentially complementary approach to the adoptive transfer of effector T cells that react with leukemia-associated antigens is to elicit responses in vivo by vaccination. While this approach may be easier to apply more broadly, it has limitations including the potential for toxicity if self-proteins are targeted and the difficulty inducing sufficiently strong T-cell responses to eliminate an established tumor burden. Adoptive transfer studies should assist in defining antigens that can be targeted safely, and the investigation of novel vaccine delivery methods may identify strategies to induce sufficiently potent responses to be therapeutically effective.

Conclusions

Studies of cellular therapy and vaccination for immunotherapy of human leukemia are just beginning. Technical and scientific obstacles need to be addressed, but our understanding of the immunologic mechanisms that may be induced to contribute to tumor eradication are now rapidly evolving. It is anticipated that these efforts will provide insights that will improve the prospects for immunotherapy as a useful therapeutic adjunct to current treatments.

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