Immunotherapy of Hematologic Malignancy

Helen E. Heslop, Freda K. Stevenson, and Jeffrey J. Molldrem

Over the past few years, improved understanding of the molecular basis of interactions between antigen presenting cells and effector cells and advances in informatics have both led to the identification of many candidate antigens that are targets for immunotherapy. However, while immunotherapy has successfully eradicated relapsed hematologic malignancy after allogeneic transplant as well as virally induced tumors, limitations have been identified in extending immunotherapy to a wider range of hematologic malignancies. This review provides an overview of three immunotherapy strategies and how they may be improved.

In Section I, Dr. Stevenson reviews the clinical experience with genetic vaccines delivered through naked DNA alone or viral vectors, which are showing promise in clinical trials in lymphoma and myeloma patients. She describes efforts to manipulate constructs genetically to enhance immunogenicity and to add additional elements to generate a more sustained immune response.

In Section II, Dr. Molldrem describes clinical experience with peptide vaccines, with a particular focus on myeloid tissue-restricted proteins as GVL target antigens in CML and AML. Proteinase 3 and other azurophil granule proteins may be particularly good targets for both autologous and allogeneic T-cell responses. The potency of peptide vaccines may potentially be increased by genetically modifying peptides to enhance T-cell receptor affinity.

Finally, in Section III, Dr. Heslop reviews clinical experience with adoptive immunotherapy with T cells. Transferred T cells have clinical benefit in treating relapsed malignancy post transplant, and Epstein-Barr virus associated tumors. However, T cells have been less successful in treating other hematologic malignancies due to inadequate persistence or expansion of adoptively transferred cells and the presence of tumor evasion mechanisms. An improved understanding of the interactions of antigen presenting cells with T cells should optimize efforts to manufacture effector T cells, while manipulation of lymphocyte homeostasis in vivo and development of gene therapy approaches may enhance the persistence and function of adoptively transferred T cells.

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I. DNA Vaccines

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DNA vaccines have now moved from an exotic possibility to practical testing in the clinic. This simple strategy to deliver selected antigens to the immune system is finding a place both in the prevention or treatment of infectious diseases, and in the therapy of cancer.[1] The overlap between infection and cancer is becoming clear, with 10%–20% of cancers, including several hematologic malignancies, such as Epstein-Barr virus (EBV)-associated lymphoma and a subset of Hodgkin's disease, arising in the context of infection.[2] Bacterial infection can also support the development of lymphoid tumors as is seen in the association between infection with *Helicobacter pylori* and gastric lymphoma.[3] Preventative vaccination could reduce the incidence of these lymphomas. At the same time, the approach to vaccination against infectious diseases is having to turn from prophylaxis to treatment of already infected individuals. This is evident from HIV infection and is gaining impetus from threats of bioterrorism with a range of organisms. The therapeutic setting is closer to the field of cancer, where, although certain tumors, such as hepatoma[4] and cervical cancer,[5] are already being prevented by prior vaccination against the associated virus, vaccination against most cancers will be as a treatment.

The aim of vaccination is to target tumor cells not eradicated by current protocols, preferably in the setting of minimal disease load. The power of the immune

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system is clear from the effectiveness of passive immunity. Monoclonal antibodies, such as anti-CD20, now have a place in treatment of B-cell malignancies.[6] Similarly, passive transfer of cellular immunity from allogeneic transplant donors can suppress leukemia via recognition of minor histocompatibility antigens.[7] The immune system is capable of attack and can maintain immune vigilance, features that could be usefully turned against cancer cells. A multiple immune attack on several target antigens should prevent the escape of tumor cells by the same principle as combination chemotherapy, but without the collateral damage. Vaccination needs to activate the appropriate effector mechanism against chosen targets. Gene-based approaches facilitate rapid testing of vaccine designs, and insertion of genes encoding additional molecules can amplify and direct immune outcome. The common ground between microbiology and cancer brings immunology back to its roots with effective vaccine development as a shared goal.

Tumor Antigens

The number of potential target tumor antigens is increasing daily, partly due to gene expression profiling and proteomics. Target antigens of hematological malignancies are expressed in different molecular forms, with distinct immune effector pathways appropriate for each (**Figure 1**). For example, glycoproteins at the cell surface, such as the surface Ig of B-cell malignancies,[8] or the clonotypic T-cell receptor of T-cell tumors,[9] are susceptible to antibody attack. However, the vast majority of potential targets arise from intracellular proteins and are expressed only as peptides associated with MHC Class I or Class II molecules. The list (**Figure 1**) includes novel or mutated peptides, the so-called cancer-testis antigens with expression limited to cancer or the testis, overexpressed autoantigens[10] and the expanding category of proteins identified by microarray analysis. Attack on these must engage CD8+ or CD4+ T cells, and for autoantigens, careful assessment of possible consequences of autoimmunity must be made. The third category of tumor antigen includes secreted proteins, with the best studied example being the clonal Ig of multiple myeloma. It is becoming clear that immune CD4+ T anti-idiotypic cells can attack MHC Class II-negative myeloma cells via an indirect process,[11] and this approach is now being tested in patients.

Gene-Based Tumor Vaccines

Gene-based vaccines have the advantage of simplicity. The concept is to take a tumor-associated gene sequence and deliver it directly to the patient, so that the gene is transcribed and translated, with subsequent presentation of the protein to the immune system in situ. Delivery is commonly via DNA, either alone or within viral or bacterial vectors. However, RNA, either total RNA from tumor cells, or specific antigen-encoding mRNA, can also be used, often transfected into dendritic cells (DCs) in vitro, for subsequent vaccination.[12] The focus of this article will be on DNA delivery since this is the approach we have taken and there are more clinical data currently available. An unforeseen benefit of DNA delivery is that the backbone of bacterial DNA has intrinsic adjuvant properties, due to the presence of unmethylated CpG dinucleotides with specific flanking motifs[13] (**Figure 2**). These bind to the Toll-like receptor 9 (TLR9) on cells of the innate immune system triggering an inflammatory response with production of an array of cytokines, including interferon (IFN)α and IFNγ. The mechanism of action of CpG has been investigated largely by the use synthetic oligonucleotides,[13] and has revealed clear differences between mouse and human responses, with TLR9 being expressed by plasmacytoid DCs in humans.[14] However, extrapolation from oligonucleotides to the action of CpG within plasmids is not straightforward, and assessment of performance of both oligonucleotides and plasmid DNA in human subjects awaits further clinical testing.
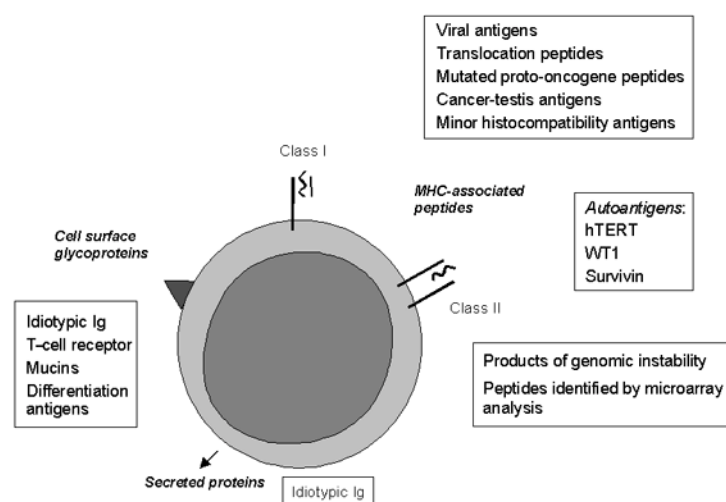
Figure 1. Target tumor antigens of hematological malignancies.

Target antigens can be expressed in 3 molecular forms: as cell surface glycoproteins, as peptides associated with the MHC Class I or Class II molecules, or as secreted proteins.

Induction of Immunity by DNA Vaccines
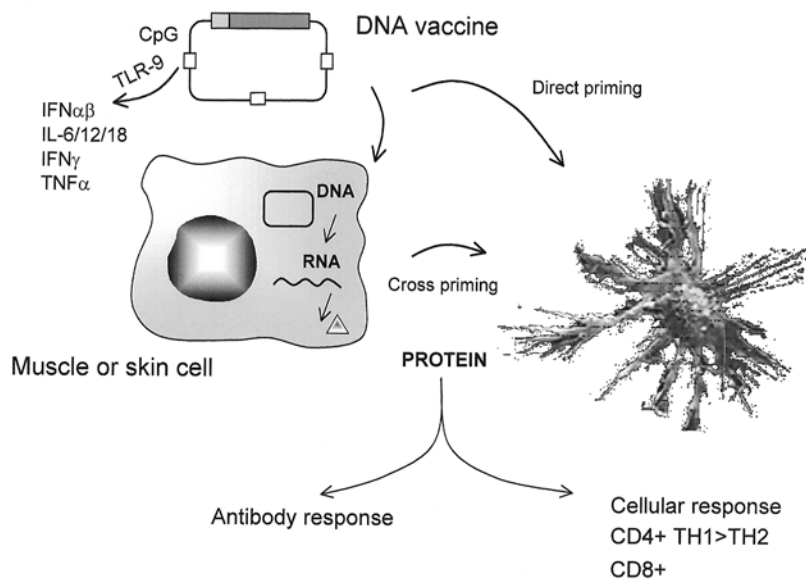
Figure 2. Pathways to induction of immunity following injection of a DNA vaccine.

Abbreviations: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor

Naked DNA with additives

DNA vaccines are usually injected into muscle or skin (**Figure 2**), with the latter often employing devices to improve efficiency of transfer, such as a gene gun to deliver DNA coated onto gold particles.[15] All arms of the immune response are activated against the encoded protein, especially CD8+ cytotoxic T cell (CTL) responses.[1] However, transfection in vivo is an inefficient process, and it appears that the amount of antigen synthesized is low. This may partially account for the relatively low levels of antibody induced as compared to exogenous protein plus adjuvant. Recent physical strategies to improve transfection rates using electroporation[16] or by formulating DNA on the surface of microparticles[17] may solve this problem. An alternative strategy is to prime the immune response by naked DNA, and then boost with antigen delivered via a viral vector, such as a replication restricted recombinant virus.[18] This generally raises the level of response, and is being tested against infectious diseases.[19] The disadvantage for cancer is that induction of immunity against the viral proteins may prevent the further boosting required to suppress tumor emergence on a long-term basis. Alphaviral vectors can be denuded of structural proteins and this may reduce vector immunogenicity sufficiently to allow repeated injections.[20]

Modified genes to engage immunity

It is relatively easy to introduce genes encoding a range of proteins aimed to promote immune recognition, such as cytokines, chemokines, complement components, and costimulatory molecules, and almost all are being

tested.[21] Targeting to professional antigen-presenting cells[22] or to B cells[23] is also being explored, as is targeting to subcellular organelles such as endosomes or lysosomes.[24,25] One problem is that it is not clear how the DNA plasmid or its encoded protein gains access to the immune system. There is evidence that muscle cells or keratinocytes provide a depot of antigen but that they are unable to prime naïve T cells directly.[26] Transfer of antigen from depots to professional antigen presenting cells, likely to be DCs, occurs via the mysterious process of "cross presentation,"[27] after which priming of T cells takes place. There may also be some direct transfection of DCs especially following injection by gene gun, but this appears very low from the intramuscular route.[28] Protein can be secreted from the depot cells but this does not generally lead to CTL responses, indicating that the cross presentation pathway involves other routes, possibly via heat shock proteins.[29] Because of this uncertainty, rational design is difficult, and the empirical approach has value. In this respect, mouse models are required to establish principles for subsequent clinical testing.

Our strategy is to use genes encoding microbial proteins to activate immunity against attached tumor proteins. This takes advantage of the fact that the immune system has been developed to fight infection, and that recognition pathways and the immune repertoire reflect this evolutionary history. The T-cell repertoire is particularly important, since vaccination against cancer is likely to have to overcome tolerance due to persistent cancer antigens. We reasoned that provision of high levels of T-cell help against a fused microbial se-

quence would substitute for the tolerized CD4$^+$ T cells, and activate linked immunity against the attached tumor antigen.[30] We chose a fragment of tetanus toxin as a safe but highly immunogenic molecule for this task. However, it is not the only pathogen-derived molecule able to act in this capacity. We have found also that a plant viral coat protein is able to promote immune responses against attached tumor antigens when delivered via DNA.[31] Since there is no preexisting immunity in human subjects against this protein, the immune outcome may be different from that using FrC.

Fusion genes in action against B-cell lymphoma

To test the approach of fusion gene vaccination, we selected the idiotypic (Id) immunglobulin (Ig) of B-cell malignancies as the initial tumor antigen.[32] One reason for this is that it is tumor-specific with no possibility of inducing autoimmunity. The second is that Id protein vaccines had clearly illustrated that anti-Id immunity could suppress tumor in mouse models[32] and in patients with follicular lymphoma.[33] DNA vaccines offered a simplified strategy for delivering Id antigens encoded by the variable region genes, V$_H$ and V$_L$. We assembled the V genes in a convenient single chain Fv (scFv) format and fused it to the Fragment C (FrC) of tetanus toxin to make DNA scFv-FrC vaccines. In mouse models this was highly effective in inducing anti-Id immunity, which was absolutely dependent on fusion between the 2 genes.[34]

A pilot clinical trial of this design in 25 patients with follicular lymphoma in first or second remission is now nearing completion. The trial is a dose-escalation from 500 to 2500 μg DNA per injection, with 6 injections into intramuscular sites over a 12-week period. No significant side effects have been observed apart from some fatigue, likely due to induction of IFNγ. All patients had pre-existing immunity against FrC due to conventional tetanus toxoid vaccination, which we predicted from mouse data would not significantly suppress the subsequent anti-Id response.[34] The FrC component of the vaccine should therefore induce a memory response. Patients with low pre-existing antibodies against FrC showed an increase after vaccination. The accompanying T-cell response as measured by proliferation on exposure to FrC was interesting in that pre-existing levels appeared to fall during the vaccination period, to be followed by expanded responses. This was seen in most patients and is likely to reflect movement of T cells to the site of injection. To date, 7/9 evaluated patients have responded to FrC, with the 2 failures being patients who had splenic involvement with lymphoma at presentation.

Responses to Id are being evaluated using recombinant scFv protein expressed in yeast cells. This ex-pression system allows folding of the protein and mediates addition of oligosaccharides to the potential glycoyslation sites which we discovered to have been introduced into the variable regions by somatic mutation.[35] The positive selection of these motifs strongly suggests that the presence of oligosaccharides in the variable region has a function in maintaining follicular lymphoma in the germinal center.[35] Proliferative responses against Id have been detected in 5/7 responders, and all patients remain in remission, with 2 patients showing resolution of small residual deposits of lymphoma by computer tomography (CT) scan. Anti-Id antibodies have not yet been measured, since a mammalian expression system is required to attach the relevant oligosaccharides and this is still being established. The results are preliminary, and will need to be compared to a parallel trial using a DNA vaccine containing tumor Id sequence linked to xenogeneic (murine) constant regions, with or without co-delivery of GM-CSF.[36] In that study, a majority of patients mounted B and/or T-cell responses to the murine Ig component, while one patient had an Id-specific T-cell response and several patients had immune responses which were cross-reactive with other patients' Id proteins. The data showed that DNA vaccination is safe and potentially a useful approach to anti-Id immunotherapy, but there is a clear need to improve performance. Whether substitution of the more immunogenic tetanus toxin sequence for the murine Ig is sufficient remains to be evaluated. Many alternative fusion strategies to promote performance of idiotypic DNA vaccines are showing efficacy in pre-clinical models, and several are likely to be tested in the clinic.[37]

DNA scFv-FrC fusion genes against myeloma

In some ways, myeloma is a unpromising disease for vaccination since patients tend to be immunosuppressed either by disease or by treatment. However, we have recently assessed responses to conventional vaccines of patients at > 15 months post autologous stem cell transplantation. We used tetanus toxoid (TT) as the vaccine, so that we could compare responses to those induced in the trial by the DNA delivered FrC. Encouragingly, we found that patients were able to respond to TT by producing antibody and proliferative T cells at this stage, indicating recovery of immune capacity even with residual tumor detectable (McNicholl et al, unpublished data). We were therefore encouraged to test the ability of the DNA scFv-FrC to induce immunity in patients. So far we have vaccinated a single patient with DNA scFv-FrC and the results are quite remarkable. We observed induction of high levels of IFNγ-producing T cells specific for Id protein purified from the

patient's serum, with no significant response against the control Id protein. Not only were these cells induced in the presence of a low level of residual paraprotein but they appear to persist for ~30 weeks after the last vaccination. Induction of IFNγ-producing T cells recognizing the FrC of TT were also observed together with an increase in the serum antibody against FrC. The effect on disease is difficult to assess, but the paraprotein has shown a slow decline since vaccination and the patient remains well. Obviously these results are preliminary but they do encourage extension of the approach to more patients in this setting.

A second, less common setting for myeloma is that of allogeneic transplantation. The strategy would then be to vaccinate the donor of the transplant with the DNAscFv-FrC derived from the patient's tumor V-gene sequences, and then transfer immunity to the patient during donor lymphocyte infusion. This has the advantage of mobilizing a healthy immune response with no tolerance anticipated. So far, we have vaccinated 1 donor and have generated proliferative T-cell responses against FrC and Id Ig, which have been transferred to the recipient patient.

DNA fusion genes to activate cytotoxic T cells

Successful induction of high avidity cytotoxic T cells (CTL) able to kill tumor cells is a goal of many DNA vaccine designs. Linkage of Fas[38] or mutant caspases[39] has been investigated as a means of increasing apoptosis and therefore antigen presentation with some success. Ubiquitin has been used to promote intracellular degradation of encoded proteins, with variable effects on outcome.[40] Conjugation with heat shock protein sequences to provide enodogenous "danger" signals[41] has also been explored. In the case of the E7 antigen of papilloma virus, HSP70 of *Mycobacteria tuberculosis* was able to amplify the CTL response.[42]

Our strategy has again been to mobilize CD4+ T-cell help by fusing a microbial sequence to the tumor antigen. However, there is a trap here which relates to the phenomenon of immunodominance in CTL responses, which has been described in mouse models.[43] The recent availability of MHC Class I peptide-loaded tetramers able to bind to specific T-cell receptors is revealing that CTL responses against viruses in human subjects show a similar phe-

nomenon, with a high degree of focusing on a limited number of epitopes.[44] The mechanism of immunodominance is still argued, but may involve killing of antigen-presenting cells by CTL induced by the most efficient peptides, before the less efficient can induce a response. With these data, it is clearly important that genes added to the vaccine to increase performance do not generate competitive peptides, which would suppress responses to tumor-derived peptides. We therefore engineered a minimized domain of FrC devoid of MHC Class I-binding motifs for mice or humans as the microbial activating sequence. In order to increase presentation of epitopes from tumor cells, we placed the epitope-encoding sequence at the 3′ end of the FrC domain.

The epitope-specific DNA vaccine is illustrated in **Figure 3**. It has been shown to induce high levels of CTL against a wide range of epitopes from mouse tumors,[45,46] and these specific CTL are capable of eliminating tumor cells even in a therapeutic setting.[46] To move to clinical application, we have used the HLA-A*0201 transgenic mouse and have demonstrated induction of CTL against peptides from viruses, tumors, and minor histocompatibility antigens. For clinical testing we have chosen to place an immunodominant peptide sequence derived from cytomegalovirus (CMV) into the vaccine. The peptide
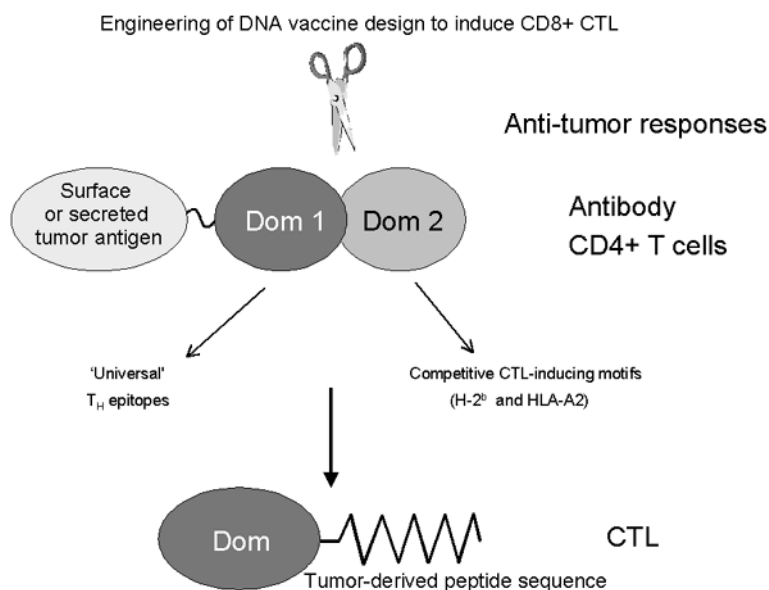
Figure 3. Modified DNA fusion vaccine to induce CD8+ T-cell responses.

The full-length FrC promotional sequence consists of 2 protein domains (Dom), Dom 1 and Dom 2. This is able to amplify antibody and CD4+ T-cell responses against fused tumor antigen. To induce CD8+ T cells, Dom 2, which contains potentially competitive MHC Class I-binding epitopes, was removed, and the candidate tumor peptide coding sequence was fused to the 3′ end of Dom 1. This engineered construct is able to induce epitope-specific CTL against a range of tumor epitopes.

is derived from pp65 of CMV[43] and the DNA vaccine is capable of inducing specific CTL in the transgenic mouse model. The danger of CMV infection or reactivation in immunosuppressed patients is high in the setting of allogeneic transplantation, especially when the donor may have no immunity against the virus. Ideally the donor should be vaccinated to protect the recipient, but there is no vaccine currently available. We are therefore testing our epitope-specific DNA vaccine for its ability to raise tetramer-positive CTL in donors prior to allogeneic transplantation. This pilot trial, necessarily restricted to HLA-A*0201 donors, has just begun.

While the trial with the DNA fusion vaccine containing a CMV-derived epitope may have immediate clinical benefit, it will also allow general insight into the performance of an epitope-specific design. This will have relevance for using tumor epitopes similarly to induce CD8^{+} T-cell attack. The eventual intention will be to combine DNA fusion vaccines to engage a wide range of immune effector pathways against multiple antigens expressed in different molecular forms by tumor cells. Not only will this raise the level of attack, but it will also prevent the notorious escape mechanisms which tumor cells commonly employ.

Concluding Remarks

Novel approaches to treatment often move from wild enthusiasm through pessimistic cynicism to eventual useful application. Mobilizing the immune system against cancer has certainly gone through these stages, and it is encouraging to see that the strategies of passive immunity, such as anti-CD20 MoAb, or graft-versus-leukemia (GVL) via allogeneic transplantation, are now in clinical practice. Active vaccination has the disadvantage of requiring a residual immune capacity in the patient, but the advantage of continuous immune vigilance once established. Genetics is providing the tools for designing vaccines to incorporate chosen tumor antigens with additional molecules to promote and direct immune outcome. Testing in mouse models will support the principles, but it is desirable to move quickly into clinical trials to put principles into practice. Fortunately, immune monitoring techniques are improving dramatically with currently available tetramer technology and cytokine measurements. Immune responses against specific antigens should precede assessment of clinical effects and allow modification of early approaches. The place of vaccination is likely to be in the setting of minimal residual disease so it is important to have measures of immune capacity following the various chemotherapeutic or MoAb treatment strategies for hematological malignancies. Gene-based vaccines are clearly able to bridge tumor antigen identification to

testing of efficacy. We believe that the incorporation of molecules able to activate high levels of T-cell help will overcome weak immunogenicity and tolerance. There will not be a "one fits all" vaccine, and the search for a universal tumor antigen may only reveal molecules against which immunity could become dangerous. It is more likely that vaccines will be either individual or aimed at small groups of patients. There will be antigens, such as cancer testis antigens, common to hematological malignancies and to other cancers. Vaccine designs will be applicable to cancer and infection. These common goals will bring together scientists and clinicians to accelerate the further development of vaccination which has been such a success in the field of public health.

II. Peptide Vaccines

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The most compelling evidence that lymphocytes mediate an antitumor effect comes from studies where allogeneic donor lymphocyte infusions (DLI) have been used to treat relapses of myeloid leukemia after allogeneic bone marrow transplantation (BMT).[1-5] Lymphocyte transfusion from the original bone marrow (BM) donor induces both hematological and cytogenetic responses in approximately 70% to 80% of patients with chronic myelogenous leukemia (CML) in chronic phase (CP).[4] A complete cytogenetic response is usually obtained between 1 and 4 months after DLI,[6] and approximately 80% of responders will achieve reverse transcriptase-polymerase chain reaction (RT-PCR) negativity for the bcr-abl translocation (the fusion product of the t(9;22) translocation found in CML) within a mean of 6 months.[6] Acute myelogenous leukemia (AML) is also susceptible to the graft-versus-leukemia (GVL) effect, with 15% to 40% of patients obtaining remission with DLI alone.[7] While significant graft-versus-host disease (GVHD) occurs in 50% of patients treated with DLI, and disease response occurs in 90% of CML patients, 55% of patients who do not experience GVHD also have disease response.[1,2] This demonstrates that GVL is separable from GVHD in some patients, and several potential antigens that drive the donor's lymphocyte response preferentially against the leukemia have been identified. There is also evidence of an autologous immune response against both CML and AML,

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directed against some of the same antigens. Remissions after DLI for AML are generally not as durable as those obtained in chronic phase CML, which may reflect the rapid kinetics of tumor growth outpacing the kinetics of the developing immune response as well as a potentially less immunogenic target cell. However, if more antigens could be determined, and if large numbers of antigen-specific CTLs could be elicited vis à vis vaccination strategies, it would allow for development of leukemia-specific therapies.

To understand the nature of vaccine-induced T-cell immunity, we will first review some of the principles of antigen recognition and highlight a recent discovery that has aided our ability to study T-cell interactions. T cells recognize peptide antigens that are present on the cell surface in combination with major histocompatibility complex (MHC) antigens. Peptides derived from cytoplasmic proteins that are 8 to 11 amino acids in length bind in the groove of class I MHC molecules and are transported via the endoplasmic reticulum to the cell surface. Larger peptides, typically 12 to 18 amino acids in length, that are derived from the processing of extracellular proteins, bind class II MHC molecules and are presented to T cells on the cell surface. Both peptide/MHC-I and peptide/MHC-II are recognized by the heterodimeric T cell receptor (TCR) on CD8 or CD4 T lymphocytes, respectively, with weak affinity and rapid off rates (**Figure 4**). Points of contact between the TCR and the peptide/MHC surface include surface amino acids contributed by the 2 alpha helical domains of the MHC molecule that flank the peptide antigen binding pocket as well as amino acids from the peptide itself.

Our understanding of the nature of antigen-specific
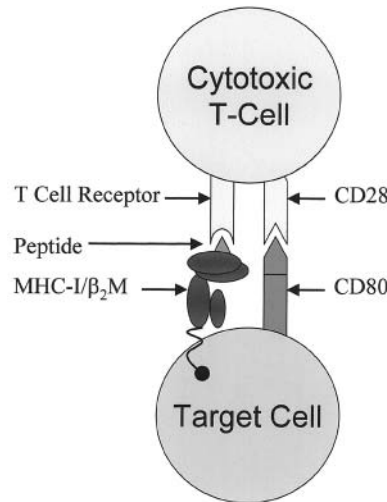
Figure 4. Two signals are required for T cell activation.

T lymphocytes reach activation threshold and are triggered to perform effector function, such as lysing target cells, after receiving two signals. The first is through the T cell receptor (TCR) and the second is through co-stimulatory receptors such as CD28.

T-cell responses has been greatly improved by the discovery that antigen-specific TCR can be reversibly labeled with soluble peptide/MHC tetramers.[8] Peptide antigen, β_2-microglobulin and the MHC-I heavy chain are folded together and, via a biotinylation signal sequence at the C-terminus of the MHC-I heavy chain, are linked covalently to streptavidin in a 4:1 molecular ratio. When the streptavidin molecule is linked to a fluorescent dye such as phycoerythrin, the resulting peptide/MHC tetramers can be used to identify antigen-specific T cells by FACS analysis because of their higher binding avidity to the cognate TCR (**Figure 5**). Using tetramers, it has been determined that up to 45% of all peripheral circulating T cells may be specific for a single dominant antigen at the height of an immune response to EBV infection[9] and similar dominance may be seen during other viral infections.[10,11] Tetramers have also
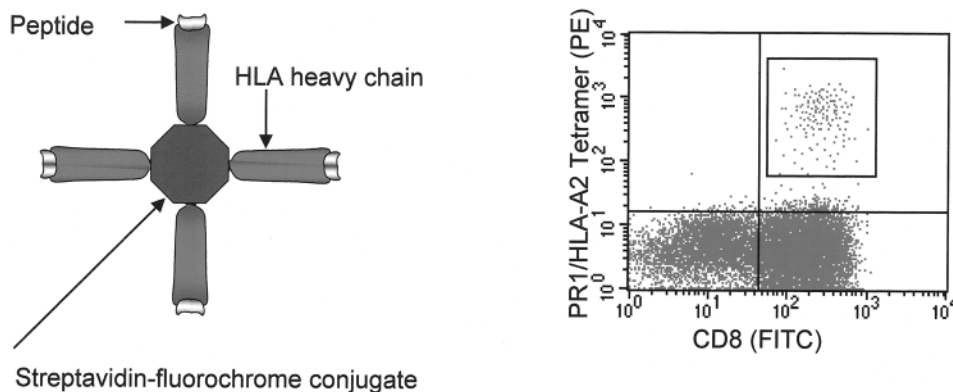
Figure 5. Antigen-specific T lymphocytes can be enumerated by peptide/MHC tetramers.

By linking four peptide/MHC monomers to a fluorochrome-conjugated streptavidin molecule, so-called peptide/MHC tetramers canb e used to label T lymphocytes that have T cell receptors (TCR) that are specific for the given peptide/MHC combination ligand. The spatial configuration of the tetramers allows for the required increase in binding avidity to stain the cells for analysis by flow cytometry. Sensitivity of this technique is often in the range of 0.01% to 0.1% of all CD8 cells.

been used to study immune responses to tumor antigens,[12] and they have also aided in their discovery.[13]

Potential Target Antigens

Various methods have been used to determine the nature of the target antigens involved in leukemia immunity. For instance, tissue-restricted minor histocompatibility antigens (mHA) that are derived from proteins expressed only in hematopoietic tissue have been shown to be the targets of alloreactive T cells.[14-18] These mHA often result from polymorphic differences between donors and recipients in the coding regions of peptide antigens that bind within the groove of MHC molecules and are recognized by donor T cells. Recently, however, a newly described mHA was found to result from differential expression in donors and recipients due to gene deletion.[14] Heterologous T-cell clones that demonstrate alloreactivity toward mHA have been established from patients with severe GVHD following BMT with an HLA-matched donor.[19-22] Some of these mHA-specific CTL clones react only with hematopoietic-derived cells, suggesting tissue specificity,[21] and therefore potentially shared antigens on leukemia. In 1 study, GVHD correlated closely with differences in the minor antigen HA-1 in HLA identical sibling transplants.[23] Expression of 2 human mHAs, identified as HA-1 and HA-2, is confined to hematopoietic tissues, and HA-2 was identified as a peptide derived from the nonfilament-forming class I myosin family by using mHA-reactive CTL clones to screen peptide fractions eluted from MHC class I molecules.[16] While this methodology has successfully defined the first CTL alloantigens, it is labor intensive and it is unclear whether CTL specific for any minor antigens identified thus far convey only leukemia-specific immunity without concomitant GVHD. Immunization of leukemia patients after allogeneic stem cell transplant (vaccination by proxy) with mHA may promote GVL and reduce GVHD if appropriate hematopoietic-restricted mHA could be targeted (such as HA-1 or HA-2). In a recent report of 3 CML patients that received DLI after relapse, however, GVHD occurred in each patient concomitant to a rise in HA-1 or HA-2-specific CTL and cytogenetic remission, albeit grade 2 or less.[24] Perhaps more importantly, a practical limit of immunotherapy targeting these mHAs is that only 10% of individuals would be expected to have the relevant HA-1 alternate allele, and < 1% would have the HA-2 alternate allele, which makes donor availability quite limiting.

An alternative immunological method to determine leukemia-specific CTL epitopes has been applied to determine whether BCR-ABL fusion region peptides could be used to elicit CML-specific T-cell responses.

Using this method, peptides are synthesized based upon an "educated guess" strategy about which proteins are potential target antigens for a selective antileukemia CTL response. The proteins are then examined for short peptides that fit the binding motif of the most common HLA alleles. These peptides are then synthesized, HLA binding is confirmed, and peptide-specific CTL responses are elicited in vitro. Since BCR-ABL is present in nearly all Philadelphia chromosome-positive CML patients, it is thought to represent a potentially unique leukemia antigen. The ABL coding sequences upstream (5´) of exon II on chromosome 9 are translocated to chromosome 22 and fused in-frame with the BCR gene downstream (3´) of exon III, resulting in the most common chimeric mRNA transcript (b3a2), which is translated into a chimeric protein (p210$^{BCR-ABL}$). Translation of b3a2 mRNA results in the coding of a unique amino acid (lysine) within the fusion region. Some HLA-A2, -A3, -A22, and B8-restricted overlapping peptides inclusive of this lysine could bind to their respective HLA alleles and could be used to elicit T-cell proliferative responses when the peptide was either pulsed onto HLA-matched normal antigen presenting cells or onto HLA-B8 positive CML cells.[25-27] However, when the b3a2 peptides were used to elicit b3a2-specific T lymphocyte lines in vitro, the resulting T cells could not specifically lyse fresh CML cells which had not previously been pulsed with the peptide.[27] This could be due to a low affinity of the peptide-specific CTL or the peptide may not be processed or presented on CML cells. More recently, however, b3a2-specific CTL were identified in the peripheral blood of chronic phase CML patients using soluble b3a2 peptide/MHC tetramers.[28] Although the tetramer-positive CTL from the patients were not examined for their ability to kill autologous CML target cells, b3a2-specific CTL elicited in vitro from healthy donors were able to kill CML cells. This suggests that bcr-abl fusion peptides may also be targets of CTL immunity.

To adapt what has been learned about immunity against solid tumor antigens to the study of myeloid leukemia antigens, we studied myeloid-restricted proteins that are highly expressed in the leukemia relative to normal hematopoietic progenitors. Myeloid leukemias express a number of differentiation antigens associated with granule formation. An example of an aberrantly expressed tumor antigen in human leukemia is proteinase 3 (Pr3), a 26-kDa neutral serine protease that is stored in primary azurophil granules and is maximally expressed at the promyelocyte stage of myeloid differentiation.[29-31] Pr3 and 2 other azurophil granule proteins, neutrophil elastase and azurocidin, are coordinately regulated and the transcription factors PU.1

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and C/EBPα, which are responsible for normal myeloid differentiation from stem cells to monocytes or granulocytes, are important in mediating their expression.[32] These transcription factors have been implicated in leukemogenesis,[33] and Pr3 itself may also be important in maintaining a leukemia phenotype since Pr3 antisense oligonucleotides halt cell division and induce maturation of the HL-60 promyelocytic leukemia cell line.[34] We have also studied another myeloid-restricted protein, myeloperoxidase (MPO), a heme protein synthesized during very early myeloid differentiation that constitutes the major component of neutrophil azurophilic granules. Produced as a single chain precursor, myeloperoxidase is subsequently cleaved into a light and heavy chain. The mature myeloperoxidase enzyme is composed of 2 light chains and 2 heavy chains[35] that produce hypohalous acids central to the microbicidal activity of netrophils. Importantly, MPO and Pr3 are both overexpressed in a variety of myeloid leukemia cells including 75% of CML patients, approximately 50% of acute myeloid leukemia patients, and approximately 30% of myelodysplastic syndrome patients.[36]

What may be critical for our ability to identify T-cell antigens in these proteins is the observation that Pr3 is the target of autoimmune attack in Wegener's granulomatosis[37] and MPO is a target antigen in patients with small vessel vasculitis.[35,38,39] There is evidence for both T-cell and humoral immunity in patients with these diseases. Wegener's granulomatosis is associated with production of *cytoplasmic* antineutrophil cytoplasmic antibodies (cANCA) with specificity for Pr3,[40] while microscopic polyangiitis and Churg-Strauss syndrome are associated with the production of *perinuclear* ANCA (pANCA) antibodies with specificity for MPO.[41,42] T cells taken from affected individuals proliferate in response to crude extracts from neutrophil granules and to the purified proteins.[38,42,43] These findings suggest that T-cell responses against these proteins might be relatively easy to elicit in vitro using a deductive strategy to identify HLA-restricted peptide epitopes. Based on this hypothesis, we identified PR1, an HLA-A2.1-restricted nonamer derived from Pr3, as a leukemia-associated antigen[13,44-46] by first searching the length of the protein using the HLA-A2.1 binding motif, the most prevalent HLA allele. Peptides predicted to have high-affinity binding to HLA-A2.1 were synthesized, confirmed to bind, and then used to elicit peptide-specific cytotoxic T lymphocytes (CTL) in vitro from healthy donor lymphocytes.

We have found that PR1 can be used to elicit CTL from HLA-A2.1+ normal donors in vitro, and that T cell immunity to PR1 is present in healthy donors and in many patients with CML that are in remission.

These PR1-specific CTL show preferential cytotoxicity toward allogeneic HLA-A2.1+ myeloid leukemia cells over HLA-identical normal donor marrow.[44] In addition, PR1-specific CTL inhibit colony-forming unit granulocyte-macrophage (CFU-GM) from the marrow of CML patients, but not CFU-GM from normal HLA-matched donors,[45] suggesting that leukemia progenitors are also targeted.

Using PR1/HLA-A2 tetramers to detect CTL specific for PR1 (PR1-CTL), we found a significant correlation with cytogenetic remission after treatment with IFN-α and the presence of PR1-CTL.[13] Somewhat surprisingly, PR1-CTL were also identified in the peripheral blood of some allogeneic transplant recipients who achieved molecular remission and who had converted to 100% donor chimerism. PR1/HLA-A2 tetramer-sorted allogeneic CTL from patients in remission were able to kill CML cells but not normal bone marrow cells in 4-hour cytotoxicity assays, thus demonstrating that the PR1 self-antigen is also recognized by allogeneic CTL.[13] These studies have established PR1 as a human leukemia-associated antigen and they established that PR1-specific CTL contribute to the elimination of CML.[13]

Recently, we found another peptide, referred to as MY4, a 9 amino acid peptide derived from MPO that binds to HLA-A2.1 and can be used to elicit CTL from HLA-A2.1+ normal donors in vitro.[47] MY4-specific CTL shows preferential cytotoxicity toward allogeneic HLA-A2.1+ myeloid leukemia cells over HLA-identical normal donor marrow.[47] MY4-specific CTL also inhibit colony-forming units granulocyte-macrophage (CFU-GM) from the marrow of CML patients, but not CFU-GM from normal HLA-matched donors. Like PR1, MY4 is therefore a peptide antigen that can elicit leukemia-specific CTL.

Several other HLA-restricted epitopes have been identified as potentially relevant leukemia-associated antigens. The Wilm's tumor antigen-1 (WT1) has emerged as a very potent immunogen containing multiple unique HLA-restricted epitopes,[48-52] and it may also be a marker of minimal residual disease since it is aberrantly expressed in both myeloid and lymphoid acute leukemia.[53-55] Various surface molecules on leukemia cells, such as CD45, present on all hematopoietic cells, and CD33 and CD19 on myeloid and lymphoid cells, respectively, have also been studied by deductive means to uncover potentially immunogenic epitopes.[56-58] While some HLA-restricted epitopes have been identified, it is unclear if any of these are leukemia-associated antigens. The method of serologic screening of cDNA expression libraries with autologous serum (SEREX) has also been used to identify

MAGE-1 and to confirm WT1 as potential leukemia-associated antigens, although there may be some controversy as to whether the MAGE proteins are expressed in leukemia blasts.[59]

In addition to these tissue-restricted epitopes in myeloid leukemias, other potential antigens that might be useful as target antigens in vaccine therapies include the idiotypes associated with lymphoid malignancies, such as immunoglobulin idiotypes and the CDR3 variable region associated with the TCR. Furthermore, antigens that are aberrantly expressed in most tumors such as telomerase and CYP1B1 contain epitopes that are recognized by CTL in vitro, which preferentially kill tumor cells, but not normal cells. Other potential targets include antigens from virus-induced hematological malignancies, such as the EBV antigens, which are discussed elsewhere.

Clinical Trials
Aside from those peptides derived from the idiotypes of lymphoid malignancies, peptides derived from the bcr-abl fusion transcript have undergone perhaps the most extensive clinical testing. The results of a previous phase I trial in CML patients showed that although a combination of fusion region-derived peptides was safe when administered subcutaneously, and immune responses could also be measured by ELISPOT after vaccination, meaningful clinical responses were not observed. More recently, the same group at Memorial Sloan-Kettering Cancer Center reported on 14 patients in a phase II study that were given 5 injections of 6 peptides over 10 weeks. A decrease in the percentage of Ph+ cells was noted in 4 patients in previous hematological remission; 3 were also receiving interferon, and 1 was receiving imatinib mesylate.[60] Transient PCR negativity was also noted in a few additional patients, although these patients had received prior allogeneic transplant, and donor lymphocyte infusions.

Because heat shock protein 70 (HSP70) is associated with antigenic peptides and is involved in chaperoning these peptides in the MHC-I antigen processing pathway, autologous cellular extracts containing HSP70-peptide complexes have been studied as a vaccine in chronic phase CML patients. At the University of Connecticut, HSP-70-peptide complexes purified from leukapheresis products were administered to CML patients that that had not yet achieved a major cytogenetic response after 6 months of imatinib mesylate treatment. Of the first 5 patients that completed all 8 weekly subcutaneous injections, major cytogenetic responses were noted in all 5, and only mild cutaneous reactions were seen.[61] Importantly, ELISPOT responses to the vaccine preparation were also noted in some patients.

Although results from both the HSP70 and the bcr-abl vaccine studies are important because they demonstrate that the vaccines can induce immune responses in CML patients and clinical responses are possible, true cause and effect has not been established since patients in both studies concomitantly received other therapies. It is therefore not possible to determine with certainty whether the vaccines contributed to the cytogenetic or molecular responses. For instance, major cytogenetic remissions after imatinib treatment continue to be observed in more than 30% of patients beyond 6 months of therapy, and small fluctuations in the percentage of Ph+ cells may be seen throughout treatment.

Clinical studies are also being conducted in Germany and Japan using WT-1 (Wilms tumor) peptides specific for the HLA-A2 and –A24 alleles as vaccines, although results have not yet been reported. In addition, peptides derived from the hTERT telomerase protein, which is widely overexpressed in leukemia, hematopoietic progenitors, and most solid tumors, are also in phase I/II trials at the University of Pennsylvania and elsewhere. Preliminary results from many of these studies should be available within the next year.

The PR1 peptide is also undergoing Phase I/II study, and the single peptide epitope is combined with incomplete Freund's adjuvant and GM-CSF and administered every 3 weeks for a total of 3 total vaccinations. Patients with AML, CML and MDS are eligible, and the first 15 patients are fully evaluable. To judge whether a clinical response was due to the vaccine, eligible patients were required to have progression, relapse, or ≥ 2nd CR (AML patients only) prior to vaccination. Immune responses, measured using PR1/HLA-A2 tetramers, were noted in 8 of the patients and clinical responses were noted in 5 of those patients. Notably, the TCR avidity of the vaccine-induced PR1-specific CTL was higher in the clinical responders than in the nonresponders, and durable molecular remissions were noted in 2 refractory AML patients that were followed for 8 months to nearly 3 years.

Conclusion
In summary, we are beginning to learn more about the nature of the antigens targeted by T cells that mediate autologous antileukemia immunity and those that are the targets of the GVL effect. Some self-antigens might also be the targets of alloreactive CTL, as we have shown for PR1. If more antigens were identified, logical immunotherapy strategies such as vaccines or adoptive cellular therapies could be tested in patients. However, obstacles to this approach remain. We must identify which of the hematopoietic tissue-restricted peptides are recognized by T cells and we must improve

our understanding of the nature of peripheral T-cell tolerance so that we might break immune tolerance to certain peptide determinants without causing potentially destructive autoimmunity. In the future, allogeneic stem cell transplantation is likely to evolve as a platform for delivering antigen-specific adoptive cellular therapies and for post-transplant vaccination strategies where donor CTL are elicited in the recipient. Both autologous and allogeneic transplant may reset T-cell homeostasis and allow a more complete T-cell repertoire to emerge postgrafting that could be further expanded selectively against tumor antigens by vaccination posttransplant, as in a vaccination by proxy therapy in the case of allogeneic transplantation.

III. T-Cell Mediated Therapies

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The concept of using cellular immunotherapy has a long history of success in animal models where a number of studies have convincingly shown that T lymphocytes recognize and kill malignant cells. However, until recently this success has not translated to human cellular immunotherapy. In the past few years, improved knowledge of the molecular basis of antigen presentation and T-cell recognition of antigen has made it clear that many tumors possess antigens that could be targets for activated T cells. While effector mechanisms of the immune system can be extremely potent after hemopoietic stem cell transplantation where small numbers of donor leukocytes can render remissions in relapsed CML[1] or eradicate EBV-LPD,[2] cancer immunotherapy has been associated with clinical response in only a limited number of patients. If a tumor is to be a target for CTL, several conditions must be met. First, the tumor must contain unique proteins capable of providing epitopes for specific immune responses. The tumor cells must also express MHC antigens, present relevant peptides frequently enough and for sufficient durations to engage responder T cells, and express costimulatory molecules such as CD28 to induce T-cell activation. The T-cell response is therefore influenced by the type

of antigen presenting cell, which determines if there is effector and memory T-cell generation or development of T-cell tolerance.[3]

Targets for Immunotherapy

In recent years, several groups have identified a number of novel immunogenic tumor proteins by screening tumor-derived expression libraries or tumor cells using autologous sera.[4] The identification of these antigens, and the mapping of specific epitopes recognized by CD4+ and CD8+ T cells, has facilitated the development of strategies designed to augment tumor antigen-specific T-cell responses.[5]

Potential antigens for targeting on leukemia cells fall into several major categories (**Figure 1**). First, many malignancies are associated with viruses, which will present unique epitopes. Epstein-Barr virus is associated with lymphoma in immunodeficient patients as well as with a subset of Hodgkin's disease and non-Hodgkin's lymphoma. Simian virus 40 has recently been reported to be significantly associated with some types of non-Hodgkin lymphoma.[6] Another category of targets is differentiation antigens that are selectively expressed in tumor cells such as Proteinase 3 (PR-3), which is a serine protease overexpressed in CML and AML progenitors[7] and WT-1, which is expressed at higher level in leukemia than in normal hematopoietic cells.[8] Cancer–testis antigens (CTAs), represented by proteins with restricted expression among tumor cells and germinal tissues including the family of MAGE genes (MAGE-1 to 10), BAGE, GAGE, SSX-1 to 9, and NY-ESO-1,[9] are also overexpressed in some hematologic malignancies.

In the setting of allogeneic BMT, alloantigens that differ between donor and recipient are targets for T-cell recognition. Differences in MHC molecules afford potential targets for recognition when BMT is undertaken with a mismatched family member or a serologically matched unrelated donor. Even when MHC antigens are identical, minor histocompatibility antigens, which are naturally processed peptides derived from normal cellular proteins, may evoke a strong MHC-restricted response when different polymorphisms are present in donor and recipient. In most cases nucleotide polymorphisms in the respective genes are responsible for immunogenicity, although a recent report details one antigen that is immunogenic because of differential expression of the protein in donor and recipient cells as a consequence of a homozygous gene deletion.[10] Several minor histocompatibility antigens have been identified, including the HA2 antigen, which encodes a member of the myosin family, and the HY antigen, which encodes a peptide derived from the SMCY

protein selectively expressed in male cells. Polymorphisms of the adhesion molecule CD31 are also a target for allorecognition, and the risk of GVHD is increased when donor and recipient have different variants.[11] In all of these cases, alloreactivity results in GVHD as well as graft-versus-leukemia reactions. However, the pattern of tissue expression of minor antigens varies, and those selectively expressed on hemopoietic cells or on particular lineages would provide specific targets for recognition. In the pretransplant setting, CTL specific for a hemopoietic antigen may also provide antileukemic activity. For example, allo-restricted CTL specific for CD45-derived peptides induce potent activity against leukemic progenitors.[12] Perhaps the most attractive target would be an antigen that was specific to the tumor cells and crucial for its function. Candiate proteins include tumor-specific proteins resulting from chromosome translocations, such as bcr/abl or proteins overexpressed in tumor cells such as telomerase.

Clinical Experience with Cellular Immunotherapy

A variety of cell types and ex vivo manipulations have been used in clinical studies of adoptive immunotherapy (**Table 1**).

Use of unmanipulated allogeneic donor T cells

Immunotherapy with T cells has been most successful in stem cell transplantation recipients, for whom the normal marrow donors have been used as the source of T cells. Adoptive immunotherapy with donor lymphocyte infusions (DLI) after allogeneic hematopoietic stem cell transplantation (HSCT) has provided an effective means of augmenting the graft-versus-leukemia response to eliminate residual disease and for the treatment of EBV-associated lymphoproliferative diseases occurring after HSCT. However, DLI are associated with a high risk of GVHD.[30] To overcome the problem of GVHD, investigators have evaluated specific subsets such as CD4-selected cells,[13] or CD8-depleted cells,[14] or functionally defined subsets such as Th2 cells.[15] Another concern with DLI is that while most patients with recurrent CML after HSCT achieve a complete remission, a smaller number of patients with relapsed acute leukemia respond.[1] The validity of many of the potential target antigens discussed in the last section has been confirmed by studies in patients with hematologic malignancies, in whom an increase in PR1-specific cells has been shown in patients with CML responding after BMT[7] and an increase in HA1- or HA2-specific T cells has been shown in patients responding to DLI.[31]

Polyclonal T cells activated ex vivo

One solution to the problem of the alloreactivity of unmanipulated T cells is to transduce the transferred cells with a suicide gene, so they can be ablated if adverse events occur. The suicide gene that has been used most frequently is the herpes simplex virus 1-thymidine kinase (HS-tk) gene, which renders transduced cells sensitive to ganciclovir. This strategy has been used in several clinical trials and has not been associated with any acute toxicity. Although alloreactive T cells appear to be sensitive to ganciclovir, a number of limi-

Table 1. Clinical studies of adoptive T-cell therapies.

Type of T Cell	Clinical Application
Unmanipulated donor T cells	Relapse of hematologic malignancy posttransplantation[1]
T-cell subsets	CD4-selected[13] or CD8-depleted[14] cells
	TH2 and TC2 cells[15]
T cells nonspecifically activated ex vivo	Donor T cells briefly activated ex vivo and transduced with a suicide gene to treat relapse of hematologic malignancy posttransplantation[16, 17]
	T cells activated ex vivo with CD3 and CD28[18]
	Expanded cytokine-induced killer (CIK) or CD8+ NK-T cells[19]
Allodepleted T cells	Postallogeneic transplant to reduce risk of relapse[20,21]
Antigen-specific CTL	EBV-specific CTL for prophylaxis and treatment of EBV lymphoma post-BMT[2,22,23] or solid organ transplantation[24;,25]
	LMP1- or 2-specific CTL for Hodgkin's disease[26]
	Leukemia-specific CTL postleukemia relapse[27]
	Minor antigen-specific CTL postleukemia relapse[28]
Chimeric receptor transduced T cell	CD20 chimeric receptor transduced T cells for NHL[29]

Abbreviations: NK, natural killer; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; BMT, bone marrow transplantation; NHL, non-Hodgkin's lymphoma

tations have been identified. These include the immunogenicity of the Tk gene product,[32] which leads to the inadvertent destruction of Tk-expressing lymphocytes, and a reduction in the immune function of gene-modified T cells.[17] An alternative "suicide gene" is a chimeric human protein expressing the Fas intracellular domain, with 2 copies of an FK506-binding protein. Transduced cells rapidly undergo apoptosis with the addition of subnanomolar concentrations of AP1903, a bivalent "dimerizer" drug that binds FK506 binding protein and induces Fas cross-linking and as this system contains only human components it should not be immunogenic.[33] Recent studies suggest the problem of activation-induced cell death depleting tumor reactive cells from the final product can be overcome by using both CD3 and CD28 for T-cell activation.[34]

CD3- and CD28-activated cells have also been administered to patients with relapsed, refractory or chemotherapy-resistant, aggressive non-Hodgkin lymphoma (NHL) following high-dose chemotherapy and CD34-selected autologous hematopoietic cell transplantation (HCT).[18] Preliminary results suggest that this approach is associated with a rapid recovery of lymphocyte counts but there are as yet no data on antitumor activity.[18]

Allodepleted T cells

An alternative approach to overcome the problem of alloreactivity is to selectively deplete the T-cell product of alloreactive cells expressing activation markers in response to alloantigen. Several studies are evaluating this strategy using an immunotoxin directed against the activation marker CD25.[20,21,35] Preclinical studies have shown that this procedure can deplete alloreactive cells while preserving T cells reactive with viruses such as CMV and EBV and tumor antigens such as PR1 and HA1.[21] In a Phase I clinical study, 15 patients were treated and early T-cell expansion was seen in patients treated at higher doses in the absence of GVHD.[20]

Antigen-specific CTLs

One way of overcoming alloreactivity and also of compensating for the low frequency of specific immune cells for many tumor antigens is to develop antigen-specific T-cell lines or clones. To generate these cells ex vivo, there must be an antigen expressed by the putative target cell and a cell that can effectively present the antigen to T cells. It is also helpful to have an immune donor, as it is difficult to generate primary T-cell immune responses ex vivo. EBV lymphoma is an excellent model to evaluate EBV-specific CTLs, as the tumor cells express all 9 latent cycle EBV antigens (including the immunodominant EBNA3 antigens), most donors are seroposi-

tive, and the lymphoblastoid cell lines generated by infecting normal peripheral blood B cells with EBV function as excellent antigen-presenting cells.

Polyclonal EBV-specific CTL, containing both CD4^{+} and CD8^{+} T cells have been administered as prophylaxis or therapy for EBV lymphoma in high-risk HSCT recipients and have survived for up to 86 months after infusion and were able to reduce the high virus load that is observed in about 20% of patients.[36] EBV-CTL also appeared to prevent progression to EBV-lymphoma, since none of 60 patients who received prophylactic CTL developed this malignancy, compared with 11.5% of controls.[2] Further, 5 of 6 patients who received CTL as treatment for overt lymphoma achieved complete remissions. In the patient who failed to respond, the tumor was transformed with a virus that had deleted the 2 CTL epitopes for which the donor CTL line was specific.[37] EBV-specific CTLs have also been administered to patients after solid organ transplant with reports of immune and clinical responses.[24,25]

A more challenging circumstance is to generate T cells specific for leukemia antigens where the malignant cell presents antigen poorly, and the putative target antigens are weak. EBV[+] Hodgkin's disease is an example of a tumor in which a more limited array of subdominant antigens is expressed. These cells have type II EBV latency, and so only express the subdominant LMP1 and LMP2 antigens. It is possible to bias the immune response toward these antigens by overexpressing them in dendritic cells.[26] A similar approach has been used to generate HA-1–specific T cells.[38]

Another possibility when target antigens are unknown is to use leukemic cells alone or cultured with dendritic cells[39] as the antigen-presenting cell. Although this technique is cumbersome, one report details a patient with relapsed CML who attained remission after infusion of leukemia-specific CTL lines.[40] Clinical studies are also under way with minor histocompatibility antigen–specific CTL.[28]

Improving Cellular Immunotherapy Approaches

Antigen Presentation

One of the limitations of adoptive immunotherapy is the lack of a convenient source of the antigen-presenting cells necessary to generate antigen specifc CTLs. A number of approaches have been explored to circumvent this requirement. These include artificial antigen-presenting cells (aAPCs) expressing ligands for the T-cell receptor (TCR) and the CD28 and 4-1BB co-stimulatory surface molecules,[41] mouse fibroblasts retrovirally transduced with a single HLA-peptide complex along with the human accessory molecules B7.1,

ICAM-1, and LFA-3[42] and beads coupled to soluble human leukocyte antigen-immunoglobulin fusion protein (HLA-Ig) and CD28-specific antibody.[43] There has also been much recent effort to identify the optimum phenotype of infused T cells. It seem likely that for optimum persistence, a product containing both effector and memory cells will be required. It will therefore be important to correlate in vivo function with the type of product generated using different sources of antigen-presenting cells.

Genetic modification of T cells overcome tumor evasion mechanisms

Tumor cells may evade a transferred T-cell response by a number of mechanisms such as downregulation of MHC and costimulatory molecules and secretion of inhibitory cytokines. Two studies have attempted to overcome inhibition of the immune response by the immunosuppressive cytokine transforming growth factor-beta (TGF-β), which is secreted by many tumors. In murine models of both thymoma and malignant melanoma, transgenic mice genetically engineered so that all of their T cells are insensitive to TGF signaling were able to eradicate tumors.[44] In a preclinical human study, EBV-specific CTLs were transduced with a retrovirus vector expressing a mutant dominant-negative TGFβ type II receptor (DNR) that prevents the formation of the functional tetrameric receptor.[45] Cytotoxicity, proliferation, and cytokine release assays showed that exogenous TGFβ that was inhibitory to wild-type CTLs had minimal inhibitory effects on DNR-transduced CTLs. If long-term murine studies show that DNR-transduced CTLs are not tumorigenic, this approach may be used in TGFβ-secreting malignancies.

Gene transfer to modify target cell recognition

As discussed above, generation of tumor-specific T-cells ex vivo is limited by the requirement for expression of an appropriate antigen by an effective antigen-presenting cell. One means of circumventing this problem is the transduction of T cells with chimeric surface proteins that transmit TCR signals in response to target cells. Such proteins are composed of an extracellular domain (ectodomain) usually derived from immunoglobulin variable chains, which recognizes and binds target antigen. This is attached via a spacer to an intracytoplasmic signaling domain (endodomain) usually the cytoplasmic segment of T-cell receptor-zeta (TCR-ζ) chain, which transmits an activation signal to the T cell. Clinical studies using such chimeric receptors targeting CD20 are under way in patients with non-Hodgkin's lymphoma[29]; CD19 is also being evaluated as a target.[46,47] Clinical trials in patients with solid tumors have

shown that T cells expressing transgenic antigen-specific chimeric receptors have limited therapeutic activity, in part because engagement of the chimeric receptor alone is insufficient to sustain T-cell growth and activation. One means of solving this problem is to transduce antigen-specific T cells rather than nonspecifically activated cells and take advantage of the costimulation provided to the native TCR by antigen. Two recent studies have investigated this possibility. Rossig et al[48] transduced a chimeric receptor specific for the GD2 antigen into EBV-specific CTLs and showed that there was stimulation of native TCR by EBV-positive LCLs, while killing of leukemia targets occurred via the chimeric GD2 TCR. Kershaw et al[49] generated dual-specific T cells by genetic modification of alloreactive T cells with a chimeric receptor recognizing folate-binding protein. An alternative means of combining an activation and a costimulatory signal is to generate a construct containing both TCR-ζ and CD28-signaling elements.[50]

Expansion of T cells in vivo

Clinical studies of adoptive transfer of activated lymphocytes to treat cancer have in many cases been limited by poor lymphocyte survival or function. An exception has been in patients after stem cell transplantation where the proliferative environment favors expansion of infused CTL. Recently, Rosenberg's group described how a proliferative environment could be artificially induced, by administration of fludarabine and cyclophosphamide.[51] Patients with advanced melanoma received lymphoreductive doses of these cytotoxic drugs and were then infused with autologous tumor-infiltrating lymphocytes. In 6 patients there was marked expansion of the infused cells associated with tumor responses: in 2 patients the tumor responses were complete, and the infused TIL came to dominate the lymphoid compartment, suggesting a relationship between cell expansion and antitumor activity. Selective expansion of infused T cells might also be obtained by using monoclonal antibodies to deplete the lymphoid compartment prior to T-cell infusion.

Conclusions

T-cell therapies have produced definitive benefits in the treatment of relapsed leukemia after transplantation and EBV-associated malignancy. However clinical studies have also identified limitations of such therapies including inadequate persistence or expansion. With increased knowledge of the optimum methodology for generation of T-cell products, identification of additional antigen targets, and optimization of gene therapy approaches to enhance the function of adoptively transferred T cells, the list of successful applications will increase.

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