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Characterizing and optimizing immune responses to leukaemia antigens after allogeneic stem cell transplantation

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Abstract

Allogeneic stem cell transplantation remains a curative treatment for haematological malignancies resistant to other treatment approaches through the unique graft-versus-leukaemia effect (GvL). However, the lack of specificity of this response results in the targeting of normal tissue, and the morbidity and mortality associated with graft-versus-host disease (GvHD). Further improvements in exploiting the GvL effect to prevent relapse in high-risk leukaemias while minimizing toxicity have focused on the use of targeted anti-leukaemic immunotherapy. These strategies include the use of vaccines against minor histocompatibility antigens (HA-1, HA-2 and H-Y) and leukaemia-specific antigens (proteinase 3, Wilms' tumour 1 and BCR-ABL), and the adoptive transfer of leukaemia-specific T cells. The unique post-transplant milieu, which is characterized by lymphopenia, regulatory T-cell depletion and the release of growth factors, offers the opportunity to promote the expansion of engrafted T cells and enhance the specific GvL response. Techniques to reduce regulatory T-cell control over T-cell responses to leukaemia antigens could further enhance GvL reactivity. Finally, these approaches to increase GvL effects would be facilitated by transplant approaches to deplete GvHD alloresponses selectively while preserving GvL reactivity.

Keywords

leukaemia; allogeneic stem cell transplant; immunotherapy; vaccine; BCR-ABL; PR1; proteinase 3; WT-1; Wilms' tumour; regulatory T cell; Treg

Allogeneic haematopoietic stem cell transplantation (SCT) for haematological malignancies was initially performed to replace diseased marrow with marrow from a healthy normal donor following myeloablative treatment for leukaemia. Today, more than three decades of clinical experience has revealed that SCT not only reconstitutes the recipient's haematopoietic system, but also mediates a powerful and potentially curative anti-

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malignancy effect, referred to as the graft-versus-leukaemia (GvL) or graft-versus-tumour effect. The anti-leukaemia effect of the graft-versus-host reaction was recognized early in murine models.[1] The initial evidence for such an effect in humans came from studies reporting that relapse rates following allogeneic transplantation were markedly less in patients who developed graft-versus-host disease (GvHD) compared with those who did not.[2,3] The anti-leukaemic effect of GvHD was further confirmed in a study of the International Bone Marrow Transplant Registry (IBMTR) demonstrating a significant GvL effect in a large cohort of transplant patients, evidenced by relapse rates that varied with different transplant settings. The high relapse rates in recipients of T-cell-depleted, compared with non-T-cell-depleted, grafts clearly illustrated the dependence of GvL on the presence of immunocompetent donor-derived T cells in the grafted marrow.[4] Further verification of the GvL effect came from the demonstration that allogeneic donor lymphocyte infusions induce remissions in patients relapsing after SCT.[5–8] These studies suggested a close correlation between the GvL-induced elimination of disease and toxicity arising from GvHD. This realization led to efforts using in-vitro-generated, leukaemia-specific T cells, adoptively transferred to SCT recipients, to target leukaemia cells more specifically without inducing GvHD. However, despite a successful demonstration of proof of principle, the approach has not been widely applied because of its impracticability.[9]

The close association found between GvHD and the GvL effect in both experimental and clinical transplantation has led to the working supposition that T cells are the central mediators of the effect. Recently, however, alloreactive natural killer (NK) cells have also emerged as GvL effectors. Since haematopoietic cells are highly susceptible targets of NK cell attack, it is no surprise that when NK cells interact with KIR-incompatible leukaemic cell targets, they exhibit strong cytotoxicity that is not observed with human leukocyte antigen (HLA)-matched or autologous leukaemic targets. The clinical importance of NK mismatching has been highlighted by the finding of very low relapse rates in acute myeloid leukaemia (AML) recipients of haplo-identical or unrelated T-cell-depleted KIR-mismatched SCT. However, the effect may be limited to myeloid leukaemias and T-cell-depleted transplants.[10–12] This chapter will mainly concentrate on the role of T cells in immune responses to leukaemia antigens.

LIMITATIONS OF GVL

Overall survival following allogeneic SCT for malignant diseases has steadily improved, largely due to reduced transplant-related mortality.[13] In contrast, risk-stratified relapse rates have not changed significantly over the last three decades.[14] Current understanding of GvL and immune reconstitution following SCT, and possible ways to exploit the immediate post-transplant period to optimize the GvL response are discussed below.

Leukaemia antigens

During a GvL response, recipient malignant cells present antigens to the donor T cells and induce leukaemia-specific T-cell expansions. However, T cells in the graft can react against recipient HLA–peptide complexes, leading to GvHD in the skin, gastrointestinal tract or liver.[15] Host antigen-presenting cells (APCs) are crucial for the induction of both GvHD[10,16–18] and GvL.[19] Furthermore, recent data have shown that donor APCs can elicit considerable GvL responses through cross-presentation of allo-antigens and tumour antigens, and may promote or sustain GvL responses by maintaining or expanding alloreactive T cells after initial priming on host APCs.[19]

Some of the antigens that drive the GvL response have been characterized and can be categorized broadly into four classes: (1) neo-antigens expressed as a consequence of chromosome translocations or mutations (e.g. BCR-ABL);[20] (2) non-alleleic normal

proteins that are aberrantly expressed or overexpressed in the leukaemia [e.g. proteinase 3 (PR3), Wilms' tumour 1 (WT1)];[21–24] (3) viral antigens (e.g. Epstein–Barr virus);[25] and (4) allo-antigens such as minor histocompatibility antigens (mHAgs; HA-1, HA-2).[26] In the context of haematological malignancies, an ideal leukaemia-specific antigen should induce a strong cytotoxic T-cell response, be expressed on leukaemic progenitors and be intrinsic to leukaemic survival, so that viable tumour escape by downregulation of the antigen cannot occur. A functional immune response would be characterized by clonal expansions of cytotoxic and helper T cells with high avidity for the antigen, diversity in their usage of T-cell receptors (TCRs), and the emergence of cells with a memory phenotype supporting the presence of durable anti-tumour immunity.

Which antigens stimulate T-cell responses to leukaemia after SCT?

The occurrence of both allo-antigens and non-alleleic leukaemia-associated antigens in the post-transplant immune environment raises the question about whether GvL responses involve both allo- and auto-antigen responses. Evidence for the role of mHAgs in GvL comes from the clinical findings that although the GvL effect can occur in the absence of GvHD, there is a tight correlation between the rate and severity of GvHD (due to recognition of host mHAgs) and the level of the GvL effect.[27] Second, the GvL effect is attenuated when the donor is an identical twin, whose T cells can react to non-polymorphic antigens but not to host mHAgs.[28] The impact of non-polymorphic antigens can be quantitated from a large study presented by the IBMTR of 2254 persons receiving HLA-identical bone marrow transplants for a number of haematological malignancies.[4] The study demonstrated a significantly higher relapse rate for patients with chronic myeloid leukaemia (CML) undergoing a T-cell-depleted sibling bone marrow transplant, irrespective of GvHD, compared with patients receiving a syngeneic transplant (relative risk of relapse 5.14 versus 2.95 for identical twins). Furthermore, a study of twins with leukaemia showed a lower risk of relapse and improved leukaemia-free survival in those receiving higher nucleated marrow cell doses (surrogate for higher stem cell doses and higher lymphocyte doses), suggesting some form of graft-mediated GvL effect which could not be explained by syngeneic GvHD.[29] Thus, clinical transplant data cannot definitively include or rule out a contributory role for non-alleleic antigens in the GvL effect.

Support for a role for both mHAgs and non-polymorphic antigens as potential target antigens for GvL activity comes from the finding that T cells specific for mHAgs and non-polymorphic self-antigens are present after allogeneic SCT and are reactive to leukaemia cells.[8,30–33]

THE EARLY POST-SCT ENVIRONMENT AND IMMUNE RECONSTITUTION

In the first few months following bone marrow or blood SCT, the immune repertoire is dominated by T cells expanding from transplanted T cells derived from the donor's peripheral blood T-cell compartment.[34,35] This consists predominantly of central and effector memory cells with a smaller population of naïve T cells and end-stage effector cells.[36] These post-thymic cells are largely responsible for the success or failure of the transplant through their impact on engraftment, GvHD, GvL and reactivating viruses. When thinking about alloreactivity, it should be borne in mind that the donor's post-thymic T-cell repertoire has already been shaped in the thymus, such that T cells of high affinity to self-antigens are depleted (negative selection), while those encountering low-affinity antigens are lost due to 'neglect' (lack of stimulation). Thus, those mature T cells with intermediate affinity for self-peptide plus major histocompatibility complex (MHC) migrate to the thymic medulla and are then exported to the periphery, where continuous low-avidity interactions with peripheral MHCs are necessary for their homeostatic maintenance.[37] In the recipient,

this mature repertoire encounters a new antigenic environment through host APCs that can drive GvHD and GvL responses.

UNIQUE ASPECTS OF THE POST-TRANSPLANT IMMUNE ENVIRONMENT

The ability of donor T cells to exert clinically effective GvL responses against leukaemia contrasts with the apparent inability of the patient's immune system to overcome the leukaemia which has escaped from immune control.[31,38–40] This observation has appropriately focused attention on T-cell responses to mHAgs as mediators of GvL activity. However, other types of protein expressed by leukaemic cells have emerged as prospective targets for a GvL effect, including non-polymorphic proteins that are overexpressed or aberrantly expressed in leukaemic cells. The inverse correlation between immunological responses against self-antigens, such as PR1 and WT1, and GvL[30–33] suggests that the allograft creates a unique immune environment favouring clonal expansion of T cells directed against leukaemia antigens. The profoundly lymphopenic environment immediately after transplant provides a favourable milieu for rapid and extensive lymphocyte expansion.[41–44] Indeed, T cell receptor V-β spectratyping reveals that in the first few months after transplant, the T-cell repertoire is oligoclonal because of selective expansion of antigen-stimulated T cells reactive against host, leukaemia and viral antigens[8,30–33] (with the potential to cause GvHD and exert GvL and anti-viral activity), despite global immunodeficiency.[45–48] Indeed, massive clonal T-cell expansions have been reported in a patient with severe GvHD in whom the T-cell compartment was nearly completely (>95%) occupied by one graft-versus-host clone.[49]

The role of lymphopenia in anti-tumour immunity in murine models was first reported in the late 1970s.[50] More recently, animal studies have shown that lympho-ablation enhances the effectiveness of adoptively transferred tumour-specific $CD8^+$ T cells.[51,52] A number of preclinical murine studies have evaluated the role of lymphodepletion combined with vaccination strategies.[53–55] The most direct evidence for the role of homeostatic T-cell proliferation in tumour eradication in humans comes from a clinical trial involving 35 patients with advanced metastatic melanomas that were refractory to conventional treatments. Patients received a non-myeloablative regimen followed by transfer of tumour infiltrating T lymphocytes directed against overexpressed self-derived differentiation antigens, permitting huge expansion of the clones with sustained regression of melanoma in 51% of cases.[56,57]

Transient lymphopenia induced by sublethal total body irradiation or other chemotherapeutic regimens is thought to enhance the efficiency of adoptive immunotherapy by altering homeostatic mechanisms that promote the expansion and stimulation of tumour-reactive effector T cells and minimize tumour-induced immune suppression. In addition to eradicating the cells that may suppress anti-tumour responses, such as regulatory T cells,[58–60] lymphoid reconstitution of either donor or host origin may overcome inherent defects in T-cell signalling, processing or presentation, may strengthen the costimulatory functions of APCs, and increase the production and availability of cytokines, such as interleukin (IL)-7 and IL-15.[61] Furthermore, lymphodepletion may serve to educate the developing T-cell repertoire to tumour antigens, and thus may be more efficacious in this environment. As reconstitution of the T-cell compartment in lymphopenic hosts is regulated by peptides occupying MHC class I and II molecules at the time of T-cell recovery, there may be an opportunity to skew the T-cell repertoire during T-cell recovery by engaging the available MHC class I and class II molecules with peptides of particular interest. It appears that naïve T cells are more sensitive to activation by weak self-antigens during reconstitution of lymphopenic hosts,[62] which may be a window during which immune tolerance may be broken. If MHC class I and class II molecules presented tumour-associated self-peptides

during a lymphopenic episode, the host may be repopulated with tumour-reactive T cells that could lead to better tumour control. Taken together, these observations imply that the first few months after transplant offer a unique environment for delivering GvL directed against both non-alleleic and allelic antigens expressed by the leukaemia.

ANTIGENS THAT DRIVE THE GVL RESPONSE

The most widely studied leukaemia antigens include the non-allelic self-antigens PR3 and WT1, the neo-antigen BCR-ABL and the mHAgs HA-1 and HA-2. Peptides derived from these proteins are currently being tested in immunotherapy clinical trials in patients with leukaemia. Therefore, this chapter will be limited to data currently available on these antigens.

WT1

WT1 is a zinc finger transcription factor that is overexpressed in most cases of haematological malignancies.[63] Cytotoxic T lymphocytes (CTLs) recognizing HLA-A*0201 or HLA-A24 restricted epitopes of WT1 selectively lyse WT1-expressing leukaemia cells whilst sparing normal progenitors.[21,24] Murine studies have demonstrated the ability of peptide- or DNA-based WT1 vaccines to reject challenge from WT1-expressing tumour cells.[24,64,65] In mice, immunization with peptide fragments of WT1 or WT1 DNA can elicit CTL responses specific for WT1 without apparent toxicity to the small number of normal tissues that express WT1, including the kidney, indicating that the WT1-specific CTLs generated in vivo in the murine model can discriminate between WT1-expressing tumour cells and WT1-expressing normal cells, resulting in the killing of tumour cells alone with no damage to normal tissues. In humans, WT1 has been shown to be naturally immunogenic with detectable responses in patients with leukaemia.[31,32,39,40]

PR3

PR3 is a 26-kD neutral serine protease stored in azurophilic granules that is maximally expressed at the promyelocyte stage of differentiation.[66] It is overexpressed in a variety of myeloid leukaemia cells including CML cells.[67] PR3 itself may also be important in maintaining a leukaemia phenotype as it has been shown that PR3 antisense oligonucleotides halt cell division and induce maturation of the HL-60 promyelocytic leukaemia cell line.[67,68] What may be critical for the ability to identify T-cell antigens in these proteins is the observation that PR3 is the target of auto-immune attack in Wegener's granulomatosis.[69] Wegener's granulomatosis is associated with production of cytoplasmic anti-neutrophil cytoplasmic antibodies with specificity for PR3,[70] and T cells taken from affected individuals proliferate in response to crude extracts from neutrophil granules and to the purified protein.[71] PR1 is an HLA-A2 restricted, nine-amino-acid peptide derived from PR3 and is a target epitope of CTLs that preferentially lyse CML cells.[23] PR1-specific T-cell responses are detectable in patients with CML and AML.[30,33,40]

BCR-ABL

CML is characterized by the presence of Philadelphia (Ph) chromosome.[72] The Ph chromosome represents a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11). The molecular consequences of this translocation are juxtaposition of the c-ABL oncogene from chromosome 9 into the breakpoint cluster region (BCR) within the BCR gene on chromosome 22, resulting in a chimeric BCR-ABL gene.[73,74] Short peptides produced by cellular processing of the fusion protein products can be presented on the cell surface within the cleft of MHC class I and II molecules, and in this form they can be recognized by T cells.[75–77] Peptides that bind with high or intermediate affinity to HLA-A3, A11, B8 and A*0201 have been identified.[20,77–79] Moreover, lysis of BCR-ABL b3a2

peptide loaded target cells by CD8+ b3a2 peptide specific T cells in an MHC class I restricted manner has been described in humans.[75,77,80,81] However, the lysis of BCR-ABL b3a2 CML cells (which present endogenously processed peptides) appears to be inefficient and not clearly demonstrable in all systems.[75,77] Mass spectrometry studies have demonstrated the presence of cell-surface HLA-associated BCR-ABL peptides previously described as binders of HLA-A*0301 in primary CML cells from HLA-A3-positive patients. In addition, these patients mounted a cytotoxic T-cell response to this peptide that killed autologous CML cells,[82] further supporting the role of BCR-ABL as a leukaemia antigen.

MINOR HISTOCOMPATIBILITY ANTIGENS

mHAgs are peptides from polymorphic intracellular proteins that are encoded by autosomal genes and genes on the Y chromosome. They are inherited independently from HLA and show broad or restricted tissue distribution. In HLA-identical donor–recipient pairs, alloreactive donor T cells may recognize mHAgs expressed on recipient cells.[26] The polymorphisms that give rise to mHAgs may affect gene expression or encode changes in amino acid sequences that result in altered binding of the peptides to MHC, contact between the MHC–peptide complex and the TCR, or differential processing of the protein.[83] Two mHAgs with haematopoiesis-restricted expression, HA-1 and HA-2, have been studied extensively and their role in GvL has been demonstrated.[8,24] T cells recognizing these antigens eliminate all haematopoietic cells from the patient, including the malignant cells, but will not interfere with donor haematopoiesis.

ADOPTIVE T-CELL TRANSFER

The adoptive transfer of antigen-specific CD8+ cytotoxic T cells against viruses and cancer is an attractive approach, and there is evidence for its therapeutic activity in humans.[9,56,84–86] However, a drawback of this approach in the clinical setting is the difficulty to produce sufficient quantities of antigen-specific T cells for subsequent infusion. The efficacy of adoptive immunotherapy is often limited by the failure of cultured T cells, particularly cloned CD8+ T cells, to persist in vivo.[87–89] The basis for the poor survival of transferred T cells has been studied extensively. One possible explanation may be related to the phenotype of T cells infused. The pool of lymphocytes from which the adoptively transferred T cells are derived include naïve T cells and antigen-experienced memory T cells that can be divided into central memory (TCM) and effector memory (TEM) subsets.[90] The memory subsets are fundamentally different with regards to their homing, proliferative capacity and effector function. In brief, TCM cells home to lymph nodes, have limited effector function but can proliferate and become TEM cells upon secondary stimulation. In contrast, TEM cells can rapidly produce effector cytokines upon antigenic stimulation, but have limited proliferative capacity and are short lived. Elegant work by Berger et al in non-human primates clearly demonstrated an in-vivo survival advantage for adoptively transferred antigen-specific CD8+ T-cell clones derived from TCM cells but not TEM cells.[91] Furthermore, most adoptive immunotherapy approaches have focused on CD8+ T cells, although it is clear that transfer of both antigen-specific CD4+ and CD8+ T-cell populations is required for optimal in-vivo efficacy.[85,86] As an alternative to the adoptive transfer of T-cell immunity at the cellular level, T-cell immunity could also be transferred at the level of the TCR. In this strategy, autologous or donor-derived T-cell populations are equipped with a TCR of defined specificity in short-term ex-vivo cultures, and re-infusion of the redirected cells is used to supply T-cell reactivity against defined antigens.[92–94] Cloned TCR genes can serve as generic reagents for treatment of patients with malignancies expressing the TCR-recognized antigen. The feasibility of TCR gene therapy was recently demonstrated in the first clinical trial in melanoma patients.[87] The retroviral transfer of a MART1-specific TCR efficiently generated MART1-specific CD8+ lymphocytes that were

used for adoptive T-cell therapy. Infused T cells expanded in vivo and engrafted at high levels in most melanoma patients. Compared with the impressive clinical response rate of conventional adoptive T-cell therapy with expanded tumour-infiltrating lymphocytes (~50% response rate), the anti-melanoma activity of the TCR-transduced lymphocytes was relatively inefficient with only two of 15 patients showing tumour regression.[56,87] This indicated that the efficiency of TCR gene therapy should be further improved to achieve better tumour protection in vivo.

PEPTIDE VACCINES UNDER CLINICAL DEVELOPMENT FOR LEUKAEMIA

BCR-ABL vaccine

Previous trials of BCL-ABL vaccination composed of a pool of six peptide fragments showed the safety of the vaccine and adjuvant. The vaccines could elicit both humoral and T-cell immune responses to BCR-ABL; however, the efficacy of the vaccine was not demonstrated convincingly.[95,96] In a subsequent, more stringent, trial using a similar peptide combination, 10 of 10 patients with stable disease on imatinib showed cytogenetic improvement, and three of five patients who achieved complete cytogenetic response had undetectable *BCR-ABL* transcripts by nested polymerase chain reaction.[97] In contrast, a recent Phase I/II study from the UK reported no molecular benefit in five patients not in major cytogenetic response at baseline. However, of the 14 patients in major cytogenetic response at baseline, 13 developed at least 1 log fall in *BCR-ABL* transcripts, although this occurred several months after completing vaccination.[98] These results suggest that clinical responses to BCR-ABL peptides can be induced in patients with CML with low levels of stable disease. These rather modest results raise concerns that the method of vaccine administration or the immune status of the treated patients may be suboptimal, or that BCR-ABL does not induce sufficiently powerful cytolytic T-cell responses to CML.

PR1 vaccine trials

PR1 is a nine-amino-acid HLA-A*0201 restricted peptide derived from PR3, shown to elicit myeloid-leukaemia-specific CTL responses that selectively kill leukaemic CD34+ cells.[22,23] PR1-specific CD8+ T cells with a memory phenotype occur at low frequencies in healthy individuals and at higher frequencies in patients with leukaemia,[30,31,33,40] suggesting that it should be relatively easy to boost these immune responses with vaccination. Highly encouraging preliminary data from a Phase I/II study evaluating PR1 vaccination in patients with myeloid leukaemias were first presented at the annual meeting of the American Society of Hematology in 2004 and an update was presented in 2007.[99] Analysis of the effectiveness of this approach in a subgroup of 20 patients with myeloid leukaemia vaccinated following SCT showed a PR1 response to vaccine in 11 of 20 (55%) patients. Nine of 11 (82%) vaccine responders compared with one of nine (11%) patients who failed to mount a response to the vaccine had clinical responses (P=0.005). Importantly, a significant PR1 response to the vaccine was associated with significantly better clinical response and longer event-free survival.[100] These encouraging results have led to the initiation of several new studies with PR1 in less advanced patients.

WT1 vaccine trials

Oka et al reported the outcome of a Phase I study of WT1-peptide-based immunotherapy in 26 patients with myelodysplasia (MDS), AML, or breast or lung cancer.[101,102] Patients received an HLA-A24 9-mer WT1 peptide in montanide adjuvant at 2-week intervals in a dose-escalation study. The vaccine was well tolerated and the only notable side-effect was profound leukopenia in two patients with hypoplastic MDS, reversed by steroid treatment, which concomitantly abrogated the WT1 T-cell response. Twelve of the 20 evaluable patients had clinical responses, including reductions in blood or marrow leukaemic blasts,

tumour size or tumour markers. Of note, increases in WT1-specific CTL frequency correlated with a clinical response.

Similarly, vaccination with an HLA-A0201 restricted WT1126 peptide in 16 patients with AML and one patient with MDS resulted in vaccine-induced WT1-specific T-cell responses in more than 60% of patients associated with clinical responses in six of 12 responders, with one patient achieving complete remission for 12 months.[103] These very promising results indicate that WT1 vaccination can induce functional CTL responses associated with clinical improvement.

VACCINATION WITH A COMBINATION OF PR1 AND WT1 PEPTIDES

Since immune responses against leukaemia are often directed against multiple antigens,[31,38,40] there is a risk that targeting a single leukaemia antigen may result in immunological pressure against expression of the parent protein, resulting in the selection of antigen-loss variants. Therefore, the authors used a combined PR1 and WT1 peptide vaccine approach in an attempt to improve the probability of generating a sustained immune response against MDS and leukaemia. Eight patients with myeloid malignancies received a single dose of PR1 and WT1 peptide vaccines. CD8+ T-cell responses against PR1 or WT1 were detected in all patients, and the emergence of PR1- or WT1-specific CD8+ T cells was associated with a significant reduction in leukaemia load as assessed by WT1 mRNA expression. However, the responses were short lived, suggesting the need for further manipulations for a sustained response.

CLINICAL APPLICATIONS OF LEUKAEMIA-SPECIFIC ANTIGEN VACCINES: THE FUTURE

While vaccines could conceivably be used to prevent myeloid malignancies, it is unlikely that vaccines alone could eliminate established disease unless combined with other treatments. This is because vaccination, while non-invasive, clinically feasible and relatively straightforward, is more likely to be effective with lower disease burden, especially as immune strategies may require a prolonged period for effectiveness. Furthermore, most leukaemia-associated antigens are self-antigens and as such are likely to induce tolerance. Combining vaccination with strategies to overcome tolerance may improve the vaccine-induced immune response.

Combining vaccines with allogeneic SCT

The finding of increased frequencies of BCR-ABL-, PR1- and WT1-specific CTLs after SCT suggests that GvL could be further enhanced by post-transplant vaccination. The transplantation of a healthy donor immune system in a leukaemic recipient offers a unique opportunity to boost GvL by also vaccinating the donor. Immediately after SCT, conditions may be favourable for antigen-specific T-cell expansion because the preparative regimen creates a lymphopenic environment causing a surge of IL-12, IL-7 and IL-15 which strongly stimulates lymphoproliferation.[104–106] T cells recently activated by antigen can be favourably boosted by vaccine during this period. The combination of the potent GvL effect of the allograft with the vaccine boost for leukaemia-specific T cells could prove to be a highly effective strategy to control refractory leukaemias. However, before vaccination can be effectively applied in SCT recipients, it will be necessary to improve methods to selectively prevent acute GvHD and to eliminate the need for post-transplant immunosuppression. The authors' group has developed a highly effective method to selectively eliminate GvHD-causing donor lymphocytes from allografts, while sparing the valuable T cells exerting GvL and beneficial anti-microbial responses. This approach, usually referred to as selective lymphocyte depletion, uses patient-derived APCs for

stimulation of donor T cells in an ex-vivo co-culture. Allo-activated donor lymphocytes are then removed by virtue of their activation status.[107] Vaccination on a background of selective depletion may overcome the need for post-SCT immunosuppression.

Combining vaccines with adoptive T-cell transfer

The efficiency of vaccination can be further increased by combining adoptive T-cell transfer with vaccination. This would involve collecting antigen-stimulated lymphocytes by apheresis before chemotherapy, and re-infusing them with further vaccination following lymphoreductive chemotherapy. Indeed, work recently published by Rapoport et al supports the feasibility of this approach.[108] They performed a randomized Phase I/II trial in 54 patients with advanced myeloma to determine whether combination immunotherapy consisting of vaccination with the pneumococcal conjugate vaccine and adoptive T-cell transfer could correct the immunodeficiency and lymphopenia induced by high-dose chemotherapy. They demonstrated that individuals who received a single early post-transplant infusion of in-vivo vaccine-primed and ex-vivo costimulated autologous T cells followed by post-transplant booster immunizations had accelerated immune reconstitution and enhanced antigen-specific CD4+ and CD8+ T-cell function in vivo.

Therefore, the authors are exploring the strategy of inducing lymphopenia (with or without allogeneic transplantation) followed by PR1 and WT1 peptide vaccination to selectively expand leukaemia-specific CTLs during the phase of lymphocyte recovery. Selection of memory T cells prior to in-vitro expansion of antigen-specific T cells may further improve the persistence of adoptively transferred T cells in vivo.[91]

CYTOKINES

Tumour responses to adoptive immunotherapy are a function not only of T-cell specificity, but also of the ability of cells to proliferate and survive following transfer. Studies employing the isolation and expansion of virus-specific T cells from cytomegalovirus- or Epstein–Barr-virus-seropositive individuals to treat viraemia or lymphoproliferative/malignant disorders in immunocompromised patients have shown that the in-vivo survival of CD8+ T effector cells is improved by the presence of CD4+ helper cells.[85,86] The addition of IL-2 to replace the requirement for CD4-mediated ‘help’ has been investigated with promising results.[89,109] Similarly, the role of other immunomodulatory cytokines (especially IL-7, IL-15 and IL-21) to support the persistence and anti-tumour effect of infused genetically modified and unmodified T cells is beginning to be the subject of clinical investigation.

OVERCOMING TOLERANCE

Peptide vaccination, while clinically feasible and relatively straightforward from a regulatory standpoint, is probably an inefficient method of boosting T-cell responses to tumour antigens. The immunogenicity of WT1 and PR1 peptide vaccination in patients with leukaemia has been clearly demonstrated; however, such responses are short lived.[110] Since most leukaemia antigens are overexpressed, non-mutated self-antigens, they tend to eliminate autoreactive T cells in the thymus during T-cell ontogeny leading to central tolerance.[111] However, tolerance to self is not absolute. Autoreactive T cells of widely varying avidities are present in the mature T-cell population,[112] and can be activated under appropriate conditions. Recent research has shown that CD4+ T cells constitutively expressing the IL-2 receptor α chain (CD25) and the forkhead/winged helix transcription factor (Foxp3) act in a regulatory capacity by suppressing the activation and function of other T cells.[113] The important role of regulatory T cells (Tregs) in controlling tumour growth was further highlighted by the demonstration that depletion of Tregs using anti-

CD25 antibodies can evoke effective anti-tumour immunity in mice.[114] Indeed, the authors' group recently demonstrated that patients with leukaemia have significantly higher frequencies of Tregs than healthy donors.[115] Furthermore, recovery of Tregs was monitored during lymphocyte reconstitution in patients who underwent T-cell-depleted allogeneic SCT, and it was found that Treg levels appear to be low following transplant or chemotherapy but expand rapidly in the first month.[115,116] These findings are consistent with work from others,[117–119] thus suggesting that recovery of Tregs may help in the maintenance of self-tolerance. Therefore, it is possible that the efficacy of cancer vaccination could be enhanced by preferential depletion of CD4+CD25+ Tregs. The recombinant IL-2 diphtheria toxin conjugate DAB389IL-2 (also known as denileukin diftitox and ONTAK, Seragen/Ligand Pharmaceuticals, San Diego, USA) has been used to eliminate CD25-expressing Tregs prior to vaccination and enhance the vaccine-induced T-cell response. Since activated T cells also upregulate their surface expression of CD25 following activation, ONTAK may also mediate the deletion of these cell populations, thereby affecting the effector immune response against vaccines. Murine studies have shown that the timing of ONTAK administration when combined with vaccination is very important in allowing selective depletion of Tregs.[120] Similarly, in patients with renal cell carcinoma and melanoma, treatment with ONTAK 1–4 days prior to tumour antigen vaccination resulted in enhanced effector T-cell responses.[121,122] Therefore, prior Treg depletion may enhance the effectiveness of adoptive T-cell therapy or vaccination.

CONCLUSIONS

Allogeneic SCT continues to play a unique role in achieving cure of haematological malignancies that are otherwise resistant to treatment. However, as alternative treatment approaches continue to improve, and increasing numbers of older patients present with leukaemia, the challenge to cure more resistant malignancies with SCT will increase. Allogeneic SCT is least effective against high-risk leukaemias, and current manipulations of conditioning regimens, post-transplant immunosuppression and donor lymphocyte infusions have probably reached their capacity to deliver GvL. To improve the 'natural' GvL reactivity of allogeneic SCT, it will be necessary to adopt new targeted treatments to further boost GvL specifically; such approaches include the use of leukaemia vaccines and the adoptive transfer of leukaemia-specific T cells. Such boosting strategies would be made more efficient if transplants could be performed after selective depletion of T cells responsible for GvHD. This would allow vaccines and leukaemia-specific T cells to be given soon after transplant without the immunosuppression normally required to prevent GvHD. Selective depletion is under clinical development as an ex-vivo manoeuvere to deplete donor T cells of unwanted alloreactivity.[118] A similar approach, where T cells destined to cause GvHD contain a viral tyrosine kinase suicide gene permitting their removal in vivo by ganciclovir, is also undergoing clinical trials.[119] Both techniques could remove the need for post-transplant immunosuppression. Once successfully assembled into a combined transplant strategy, these novel techniques promise to significantly reduce the risk of leukaemia relapse after SCT.

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