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Post-transplant adoptive T-cell immunotherapy

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Abstract

Immune reconstitution following haematopoietic stem cell transplantation (SCT) is an often slow and incomplete process that leads to increased risk of infection and malignant disease. Immunization in SCT is frequently unsuccessful due to the prolonged lymphopenia, especially of CD4 T cells, seen following transplant. The transfusion of T cells, also called 'adoptive T-cell therapy', has the potential to enhance anti-tumour and overall immunity, and augment vaccine efficacy in the post-transplant setting. Recent advances in tissue culture, cellular immunology and tumour biology are guiding new approaches to adoptive T-cell therapy. This chapter will discuss the challenges that face the field before adoptive T-cell therapy can be translated into routine clinical practice.

Keywords

T cell; immune reconstitution; lymphopenia; haematopoietic stem cell transplantation; immunotherapy

Stem cell transplantation (SCT) has a well-established role in the treatment of haematological malignancies. Both autologous and allogeneic SCT have demonstrated efficacy as therapy for patients with leukaemia, multiple myeloma and lymphoma. Additionally, the use of SCT is being explored for patients with autoimmune diseases. The post-transplant period is characterized by a prolonged period of immunodeficiency, leading to increased vulnerability to infection.[1–3] In one study, the majority of allograft recipients experienced at least one late infection (>50 days to 2 years) after transplant.[4] Multivariate analysis showed that infection was the dominant factor associated with non-relapse mortality. In patients with chronic myeloid leukaemia, transplantation with a T-cell-depleted graft has been associated with an increased risk of relapse.[5] Several authors have reported a correlation between higher absolute CD4 and CD8 lymphocyte counts and improved disease-free and/or overall survival.[6–8] Even after lymphocyte numbers recover, lymphocyte function is often impaired.[9–11] These observations reinforce the importance of immune reconstitution in the overall effectiveness of transplantation.

Adoptive immunotherapy is the isolation, ex-vivo activation and infusion of antigen-specific or non-specific lymphocytes. Adoptive cellular therapy can be considered as a strategy aimed at tumour elimination through direct anti-neoplastic effects, or through indirect effects mediated by immunity directed against elements supporting tumour growth such as

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angiogenesis. Adoptive cellular therapy using autologous or allogeneic cell infusions may also have a role in replacing, repairing or enhancing the immune function damaged as a consequence of cytotoxic therapy. Analysis of the presently available clinical results suggests that, despite some disappointments, there is room for optimism that both adoptive immunotherapy and active immunotherapy (vaccination) may eventually become part of the therapeutic arsenal to prevent or combat cancer in a more efficient way. This chapter will describe the background, rationale, and current clinical use and experimental approach of adoptive cellular therapies to improve immune reconstitution in the setting of SCT for haematological neoplasms.

IMMUNODEFICIENCY FOLLOWING HAEMATOPOIETIC STEM CELL TRANSPLANTATION

In addition to compromising the ability of SCT patients to mount effective anti-tumour immune responses, post-transplant immune suppression clearly increases the risk for serious infections with varicella-zoster virus, cytomegalovirus and *Streptococcus pneumoniae*.[12,13] Early recovery of lymphocytes and lymphocyte function has been linked to improved survival following both auto- and allotransplantation.[6,14] In the immediate post-transplant period, lymphocyte restoration is achieved by expansion of mature T cells present in the graft, and not de-novo production from the thymus or bone marrow.[15–17] CD4+ T-cell regeneration occurs via a thymus-dependent mechanism, while CD8+ T-cell regeneration occurs via a thymus-independent pathway.[10,18] Therefore, after transplant, there is a prolonged deficiency of CD4 + T cells compared with CD8+ T cells, particularly in older patients, secondary to limited thymic regenerative capacity.[17,19] While younger patients eventually recover thymic output, the thymic deficiency seen following transplantation may not be fully reversible in older patients.[20,21]

The CD4+ T-cell deficiency noted after transplant is particularly significant as several studies have demonstrated the importance of these cells in the stimulation of CD8+ T cells and the enhancement of antibody production by B cells. CD8+ T cells that engage antigen in the absence of CD4+ T cells develop normally but do not proliferate well and do not persist, becoming so-called ‘helpless T cells’.[22] This phenomenon may be responsible for the poor cytotoxic CD8+ T-cell responses seen in human immunodeficiency virus (HIV),[23] and could be operative in transplant patients. In addition to providing a critical stimulus for CD8+ T cells, CD4+ T cells are required for maximal antibody production.[24] The importance of CD4+ T cells has been demonstrated in humans where responses to immunization and severity of infection have been correlated with CD4 counts specifically,[25,26] and survival correlates with CD4 counts in patients after infection with HIV.[25]

Humoral immunity recovers more quickly than cellular immunity in the immediate post-transplant period; however, immunoglobulin subset levels are often suppressed such that protective immunity is compromised, and response to vaccination remains a real problem in SCT patients.[27] In the setting of allogeneic SCT, immunization of the donor has led to increased titres of *Haemophilus influenzae* type B and tetanus toxoid antibodies.[28,29] Similar findings were noted in patients undergoing autologous SCT who underwent pre-transplant immunizations.[30] In this age when so many promising tumour vaccines are in clinical trials, strategies to optimize responses to vaccination in SCT patients have become increasingly important.

ALLOGENEIC T-CELL THERAPY

The first form of human adoptive T-cell therapy was the passenger T cells present in stem cell infusions from bone marrow harvests. In a retrospective analysis, Weiden et al showed that the probability of tumour recurrence was lower for allogeneic transplant recipients compared with

those who received syngeneic grafts.[31] It is now well established that allogeneic SCT and donor lymphocyte infusions (DLIs) are an effective, but potentially toxic, form of non-specific immunotherapy in chronic myeloid leukaemia,[32] although clinical responses for other malignancies have been disappointing.[33,34] Early studies showed that the infusion of donor T cells soon after a myeloablative transplant conditioning regimen resulted in the marked augmentation of acute graft-versus-host disease (GvHD).[35] In the setting of allogeneic haematopoietic SCT, Kernan et al have shown that the initial dose of infused T cells and the time from initial transplant have a major effect on the incidence and severity of acute GvHD.[36] It has only recently been appreciated that the incidence of acute GvHD may be lower following the infusion of donor T cells in the setting of non-myeloablative SCT.[37] Studies by Dazzi et al show that in the steady-state setting of relapsed chronic myeloid leukaemia, infusions of resting T cells result in a decreased incidence of GvHD when given by dose fractionation, starting with low doses of donor cells and escalating subsequent doses as required.[38] In a non-randomized trial, they compared a bulk, single DLI (average 1.5×10^8 $CD3^+$ cells/kg) with an escalating dose regimen of DLI, where increasing numbers of cells (average total 1.9×10^8 $CD3^+$ cells/kg) were given at 20-week average intervals between infusion. They found that anti-leukaemic effects were preserved but that the incidence of GvHD was much lower using the escalating dose regimen of DLI. The authors have recently reported on a Phase I trial where patients with aggressive haematological malignancies received induction chemotherapy and conventional DLI (median 1.5×10^8 mononuclear cells/kg), followed 12 days later by ex-vivo, CD3/CD28-activated DLI (aDLI). aDLI was dose escalated from 1×10^6 to 1×10^8 CD3+ cells/kg in five levels. Out of 17 evaluable patients, seven developed acute GvHD and four developed chronic GvHD. Eight patients achieved complete remission. This study demonstrated that adoptive transfer of costimulated activated allogeneic T cells is feasible, does not result in excessive GvHD, and may contribute to durable remission in diseases where conventional DLI has been disappointing.[39]

PRINCIPLES OF T-CELL GROWTH

Adoptive cellular therapy depends on the ability to optimally select or genetically produce cells with the desired antigenic specificity, and then induce cellular proliferation while preserving the effector function, engraftment and homing abilities of the lymphocytes. Unfortunately, many previous clinical trials were carried out with adoptively transferred cells that were propagated in what are now understood to be suboptimal conditions that impair the essential functions of the adoptively transferred cells. Our greater understanding of the process of T-cell activation mediated through cell surface receptors and proteins now indicates that this is a complex multistaged process of recognition, adhesion and stimulation. In vivo, the generation of antigen-specific T cells requires the interaction of dendritic cells (DCs) and naïve T cells in a secondary lymphoid organ, usually a lymph node.[40]

For over 50 years, immunologists have sought to understand how the balance between immune activation and self tolerance is induced and maintained. Bretscher and Cohn first proposed a two-signal model of B-lymphocyte activation that was later modified by Lafferty and Cunningham for T-cell activation and allograft rejection.[41] The essential features of these models were that activation of lymphocytes requires an antigen-specific signal, termed ‘Signal 1’, as well as a second antigen-non-specific event, termed ‘Signal 2’. Moreover, these theories and later modifications proposed that Signal 1 in the absence of Signal 2 led to tolerance or apoptosis. Indeed, in some instances, the binding of tumour antigen to the T-cell receptor in the absence of costimulation not only fails to activate the cell but also leads to functional inactivation.[42] It is now appreciated that antigenic stimulation of T cells leads to at least three distinct outcomes: (1) activation, clonal expansion and differentiation to produce cells that secrete distinct subsets of cytokines or to express lytic machinery; (2) induction of an unresponsive state termed ‘anergy’; and (3) induction of apoptosis.[43,44]

Striving for optimal T-cell function

T cells exist in several distinct stages of differentiation. Naive $CD4^+$ and $CD8^+$ T cells undergo unique developmental programmes after antigen activation, resulting in the generation of effector memory and long-lived central memory T cells (T_{EM} cells and T_{CM} cells, respectively). Three models by which memory $CD8^+$ T cells can be generated have been proposed. In the linear differentiation model, an autonomous antigen-triggered differentiation process consisting of conversion from naïve to effector to T_{EM} cell occurs, followed by the appearance of T_{CM} cells after antigen clearance through a process of dedifferentiation.[45] The signal strength model proposes that naïve T cells progress through hierarchical thresholds for proliferation and differentiation as the strength and duration of the interaction with antigen-presenting cells (APCs) is increased.[46] T cells receiving the weakest signals do not survive, whereas high-intensity signalling causes the development of terminally differentiated effector T cells that cannot survive into the memory phase. The T_{CM} cells, being the least differentiated of the antigen-stimulated T cells, retain the developmental options of naïve T cells, including their capacity for marked clonal expansion. The stem cell model proposes that the cells within the T_{CM} cell compartment are self-renewing and serve as a source of effector T cells.[47] Although the details and mechanisms of differentiation remain to be clarified, it is clear that the various T-cell memory subsets have specialized roles and that not all the subsets would be efficacious in the setting of adoptive T-cell therapy for the treatment of cancer.

At present, naïve T cells are not thought to be useful for adoptive transfer as they are unable to kill tumour cells. The demonstration by Sallusto et al[48] that T_{CM} and T_{EM} cell subsets have discrete trafficking and functional properties has the potential to fundamentally alter approaches to adoptive T-cell therapy. In retrospect, previous clinical trials have primarily tested adoptively transferred populations of $CD8^+$ T_{EM} cells.[49] This approach was taken because available tissue culture technologies resulted in rapid differentiation of T cells to late-stage effector cells; late-stage T_{EM} cells express CD57 and have poor replicative capacity.[50,51] In vitro, T_{EM} cells are superior to T_{CM} cells at tumour cytotoxicity. However, in vivo, T_{CM} cells exhibit superior therapeutic effects when compared with T_{EM} cells on a per cell basis.[52] Therefore, in principle, adoptive T-cell transfer strategies are attractive for the ability to generate long-lived populations of central memory T cells capable of immunosurveillance as well as tumour eradication, which is why efforts to test T_{CM} cells in clinical trials are currently of high priority.

Factors determining the optimal $CD8^+$ T cells for adoptive transfer

The cellular basis of immunological memory has been one of the central issues of immunology for more than 50 years. Many studies in mice indicate that true memory with long-lived T cells is only established in the absence of persistent antigen exposure.[53,54] This raises a paradox for tumour immunologists regarding whether and how central memory can be established in tumour-bearing patients. Relevant to this issue is that in patients at risk of developing Epstein-Barr virus lymphoma, adoptively transferred gene-marked cytotoxic T lymphocytes (CTLs) persist for years,[55] demonstrating that, in principle, it is possible to establish T_{CM} in cancer patients by adoptive transfer. Therefore, to understand approaches to generate persistent immunity in patients where tumour antigens are unlikely to be eliminated completely, it might be more instructive to study the human immune response in patients with chronic persistent viral and parasitic infections.[56,57] A major issue of relevance is the differentiation pathway leading to T_{CM} cells. Some studies have indicated that naïve T cells first differentiate into effector T cells, a proportion of which then differentiate into T_{CM} cells.[58] In contrast to this linear differentiation model, other studies have suggested that parallel differentiation occurs, with naïve T cells differentiating directly into T_{CM} and effector cells simultaneously through asymmetric division.[59,60] Resolution of the above issues is important so that culture systems can be devised to optimally derive populations of T_{EM} and T_{CM} cells.

In humans, adoptive T-cell therapy approaches to date have used peripheral blood, the tumour bed, malignant effusions and draining lymph nodes as the anatomical source from which T cells are derived for adoptive transfer.[61–64] Given the recent demonstration that bone marrow is a major reservoir of self-reactive $CD8^+$ T cells,[65] it is important to determine if improved anti-tumour effects are observed when bone-marrow-resident T cells are used for adoptive transfer. The bone marrow of breast cancer patients has been shown to contain $CD8^+$ T cells specific for peptide epitopes from the tumour antigens MUC1 and Her-2/neu,[66] and adoptive transfer of bone-marrow-derived human $CD8^+$ memory T cells mediates anti-tumour activity in mice bearing tumour xenografts.[50] Furthermore, bone marrow from patients with either pancreatic cancer or myeloma has also been shown to be enriched with tumour-reactive $CD8^+$ T cells.[67–70]

A role for $CD4^+$ T cells in adoptive T-cell transfer

Many studies have shown that the generation and/or maintenance of $CD8^+$ T-cell memory requires $CD4^+$ T-cell help,[71] and that tumour-specific immunity is enhanced with $CD4^+$ T-cell help, particularly in the absence of tumour expression of major histocompatibility complex (MHC) class II molecules[72] as is commonly observed with aggressive haematological malignancies. Adoptively transferred $CD4^+$ T cells have the potential to augment tumour immunity by several mechanisms that might enhance the survival and function of $CD8^+$ T cells, including the secretion of essential cytokines such as interleukin (IL)-2 and IL-21,[73] and the expression of CD40L.[74] Besides their intimate involvement in priming tumour-specific CTLs, $CD4^+$ cells participate in additional effector functions. Evidence indicates that other cytokines produced by $CD4^+$ T cells can recruit and activate macrophages and eosinophils that, in turn, mediate anti-tumour effects.[75] Clinical adoptive transfer studies also show that the persistence of adoptively transferred cytotoxic $CD8^+$ effector T cells is enhanced with the concomitant administration of IL-2[76] or $CD4^+$ T cells.[77] Recent studies in patients with myeloma show that the adoptive transfer of mixed populations of pathogen-specific $CD4^+$ and $CD8^+$ T cells promoted the establishment of immunity with a robust central memory component.[78] However, it is not yet known if this approach enhances the establishment of immunity to self-antigens in cancer patients.

T-cell development is highly dependent on the nature of the cytokine milieu, although species-specific differences exist relating to the primacy of individual cytokines in this process. The common gamma chain (γ_c) is a shared receptor component for the IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 receptors. In the mouse, IL-15 is not produced by T cells, whereas human $CD4^+$ memory T cells are reported to constitutively produce and proliferate in response to IL-15,[79] suggesting different roles for IL-15 in mouse and human $CD4^+$ memory T cell homeostasis.[80] Genetic analysis of patients with a γ_c deficiency indicates that the human is absolutely dependent on cytokines that signal through γ_c for T-cell development, as T cells are absent in these individuals, whereas in γ_c-deficient mice, the spleens of older mice have nearly normal numbers of $CD4^+$ T cells,[81] indicating that γ_c-independent signals can support $CD4^+$ T-cell development in mice but not humans. In mice, adoptively transferred $CD8^+$ T cells cultured in IL-15 show more tumour cytotoxicity than those cultured in IL-2,[49] so the use of IL-15 for adoptive T-cell therapy in humans holds substantial promise to augment T-cell numbers and effector functions, although species differences can be expected.

At present, one of the most important issues facing the field is the complexity of $CD4^+$ lineage T cells. Until recently, T cells were separated into two different subsets named Th1 and Th2 cells, based on the pattern of cytokines that they produce when stimulated. Regulatory T cells (Tregs) represent a subset of thymic-derived T cells that exert an immunosuppressive influence and are thought to be essential for mediating tolerance. Tregs express IL-10 and tumour growth factor-β, but may exert inhibitory effects via direct cell contact. Several types of $CD4^+$ Treg

cell have now been described in humans,[82] and it is probably important to remove Treg cells from adoptively transferred T-cell populations because they suppress anti-tumour immunity.[83] Since Treg cells are often enriched in tumour-infiltrating lymphocytes and the peripheral blood in cancer patients,[84] it is possible that the outcome of previous adoptive T-cell therapy clinical trials was compromised because the adoptively transferred T-cell populations inadvertently contained Treg cells.

Recently, a fourth axis to the $CD4^+$ T-cell lineage was recognized, as a new IL-23-driven subset of IL-17-producing $CD4^+$ T cells called 'Th17' has been described.[85] Th17 cells are pro-inflammatory in some settings, but paradoxically may exert an immunosuppressive effect in the setting of malignancy. An interesting finding is that tumours express IL-23, which may promote the expansion of Th17 cells, resulting in decreased immunosurveillance of tumours by preventing Th1-cell-driven $CD8^+$ T-cell infiltration.[86] However, as evidence from several groups indicates that IL-17-producing Th17 cells represent the key effector cells in the induction of autoimmunity, rather than, as once was thought, interferon-γ-producing Th1 cells,[87] it remains possible that adoptive transfer of Th17 cells might augment anti-tumour responses. Thus, early in the evolution of tumours, Th17 cells may have a pro-carcinogenic effect, while Th17 cells may have a pharmacological ability to treat established tumours.[88]

DEVELOPING OPTIMAL CELL CULTURE SYSTEMS

The only form of adoptive cellular therapy routinely employed in the practice of medicine is allogeneic bone marrow or peripheral blood SCT.[89] In this setting, the establishment of donor haematopoiesis results in a graft-versus-leukaemia (GvL) effect mediated by donor lymphocytes. The administration of donor leukocyte infusions in the post-transplant period has also been shown to result in the regression of persistent or recurrent disease.[90] The adoptive transfer of donor (allogeneic) T cells undergoing ex-vivo activation has promise to augment this effect.[39,91] Ex-vivo culture approaches to alter the ratio of effector T cells and Tregs have the potential to decrease the risk of GvHD while preserving anti-tumour effects.[92] A clear separation of GvHD and GvL has been demonstrated by the infusion of Tregs in leukaemia-bearing mice.[93] However, whether this strategy will work in humans is unknown. Since allogeneic transplantation is associated with significant treatment-related morbidity and mortality, adoptive transfer of tumour-specific autologous T cells has been examined. A central issue for the development of clinical adoptive T-cell therapy strategies has been the development of culture systems in order to produce adequate numbers of effector T cells for autologous therapy.

Two basic approaches are being tested for clinical adoptive T-cell therapy. The first approach has been to isolate and activate in-vitro antigen-specific T cells from peripheral blood or tumour specimens, and then to use repetitive stimulation to clonally expand the antigen-specific T cells in vitro by various approaches. In the second approach, polyclonal ex-vivo activation of the T cells is done based on three assumptions: first, tumour-specific T cells are present in the patient; second, the tumour-specific T cells have been primed in the patient; and third, the in-vivo function of the tumour-specific T cells in the patient is impaired. In the second approach, the cells are activated in a polyclonal fashion by various means in vitro, and are then re-infused into the patient with the expectation that they will respond directly to the tumour or to tumour antigens presented by APCs. The first approach guarantees antigen specificity but is costly and labour intensive, and the second approach is technically more rapid and feasible. In practical terms, only the second approach has been sufficiently robust to support randomized clinical trials.[64,78] The rationale for the second approach has been strengthened substantially by the realization that many patients are already primed to their tumour,[94–96] and that the major challenge is improving the quality and quantity of the natural immune response.[97] However,

interest in both approaches has been reinforced by the realization that antigen-independent expansion of the transferred memory T cells can occur in vivo[98] under certain conditions.

Strategies to present antigen to T cells ex vivo

The most appropriate methods of ex-vivo T-cell culture mimic the physiological processes whereby DCs generate a constellation of antigen-specific and costimulatory signals in the T cells. DCs are the most efficient APCs for the activation of naïve T cells. However, although useful for therapeutic vaccination, the generation of DCs is laborious and expensive, and yield and potency vary between patients. Therefore, DCs may not be optimal as APCs for large-scale adoptive T-cell therapy trials. In addition, DCs have limited replicative potential; for ex-vivo expansion of autologous T cells, it is desirable to have APCs with extensive replicative potential to facilitate both the scale-up of the process and multiple rounds of T-cell stimulation. As noted previously, since many patients are already primed to their tumours, other less potent forms of APC might suffice to induce T-cell activation. The best results to date have been with the rapid expansion method developed by Riddell and Greenberg, which uses irradiated allogeneic peripheral blood mononuclear cells as APCs (also known as feeder cells) to expand CTLs for adoptive transfer.[99] The main limitation of this approach is in scale-up. Schultze et al have shown that CD40-stimulated B cells, which have extensive replicative potential, are an efficient means to propagate antigen-specific T cells.[100] Thus, while currently available tissue culture approaches have provided proof of concept for adoptive therapy, a current priority is to develop alternative approaches that can support large-scale trials.

To generate antigen-specific T cells, cell lines and beads can be engineered to create artificial APCs and avoid the need to use autologous APCs for patient-specific cultures.[101] General approaches have been to produce artificial APCs, either by coating beads with CD3-specific antibody or peptide–MHC complexes, or by transfecting cells that lack endogenous MHC molecules with MHC molecules and costimulatory molecules. Enhanced polyclonal T-cell activation and proliferation results when cells are stimulated through T-cell receptors and CD28.[102] In addition, CD28 stimulation maintains telomere length in human T cells, and this might improve engraftment and persistence of adoptively transferred T cells.[103,104] This culture system has been adapted for clinical use, and starting with an initial apheresis product, it is possible to generate the number of mature T cells found in an adult within 2 weeks of ex-vivo culture.[105,106]

Magnetic beads coated with MHC class I molecules loaded with specific peptide have been used to elicit antigen-specific T-cell propagation.[107] Following isolation and expansion, cell populations generated using such beads specifically kill antigen-expressing target cells in vitro, and display anti-viral therapeutic effects in rodents.[108] Others have used non-magnetic microspheres coated with complexes of recombinant peptide-loaded MHC molecules to successfully generate CTLs ex vivo from naïve precursors.[109] Peptide–MHC tetramers presenting peptides from the melanoma tumour antigens melanoma-associated antigen recognized by T cells 1 (MART1) and glycoprotein 100 (gp100) have also been used to isolate high-avidity tumour-reactive $CD8^+$ T cells from a heterogeneous population by flow cytometry. The tetramer-reactive cells could be cloned and retained their functional activity upon re-stimulation.[110,111] Latouche and Sadelain have engineered APCs that could be used to stimulate T cells of any patient expressing a specific human leukocyte antigen (HLA) allele.[112] Mouse fibroblasts were transduced retrovirally with a single HLA class I complex along with the human accessory molecules CD80 (also known as B7-1), CD54 (also known as ICAM-1) and CD58 (also known as LFA-3). These artificial APCs consistently elicited and expanded CTLs from patients of the appropriate haploype specific for MART1 and gp100. The authors have also found that artificial APCs that express 4-1BB ligand efficiently expand human central memory $CD8^+$ T cells that have potent cytolytic function,[107,113,114] and

others have shown that CD83 expression on artificial APCs enhances the generation of CTLs.[115]

ADOPTIVE CELLULAR THERAPY: DOSE AND SCHEDULING ISSUES

Information on the dose and schedule dependence of adoptively transferred cells is widely scattered in the literature, such that there is no standardized system of administration. There is evidence in non-lymphopenic hosts suggesting that fractionated doses of adoptively transferred T cells are superior to a single infusion of T cells.[116] The ideal dose of transferred cells is related to the tumour burden, and the homing and persistence (memory) characteristics of the infused cells.[117] Doses of adoptively transferred cells are usually reported as the total number of viable cells administered, or as the total number of viable cells per kg body weight or per square meter body surface area. However, total lymphocyte numbers do not correlate well with body surface area, but rather display a stronger inverse correlation with age. Other variables add to the complexity, particularly the fact that, in the case of T cells or other adoptively transferred cells with high replicative potential, the infused dose may not relate well to the steady-state number of cells. Therefore, dose considerations are more complex than in other areas of transfusion medicine, where, for example, the maximal level of transfused red cells or platelets occurs immediately following infusion. Several recent studies have shown that severe lymphodepletion of the patient prior to cell administration can create optimal conditions to promote anti-tumour effects, and post-transplant adoptive T-cell transfer can take advantage of the homeostatic proliferation that occurs during this time.[118]

Schedule-dependent efficacy and adverse effects from adoptively transferred cells have been reported. Many studies in rodent tumour models show that the administration of cytotoxic therapy can enhance the effects of adoptively transferred cells through mechanisms that are independent of direct tumour cytoreduction. In studies with cyclophosphamide, enhancement of cellular immunotherapy is due to multiple effects, including: (1) killing of host regulatory lymphocytes that suppress anti-tumour immune responses;[119,120] (2) creating ‘space’ in the host so that the adoptively transferred cells can engraft;[117] and perhaps (3) enhanced cross-priming of tumour antigens. In contrast, it is probably best, when feasible, to harvest autologous T cells for ex-vivo expansion prior to initiation of chemotherapy. This is because adults have limited regeneration of T cells from the thymus, and therefore, the repertoire remains contracted for long periods of time and, in many cases, never recovers.[19,121] Naïve T cells are most sensitive to the effects of cytotoxic chemotherapy, and their numbers are severely depleted in heavily pretreated patients. It is not yet known whether the tumour-specific T cells are derived from primed or naïve T cells in the host, and this likely varies depending on the intrinsic immunogenicity of the tumour. Studies with tetramers binding tumour peptides presented in the context of MHC molecules show that, in some patients, chemotherapy can ablate the tumour-specific T cells that have an effector phenotype, while sparing memory cells.[122] The mechanism for this observation is unknown, but the authors speculated that the effector cells were in the active phases of the cell cycle, and were therefore rendered relatively susceptible to the cytotoxic effects of chemotherapy. If these results are confirmed, they would argue that patients should have their repertoire ‘archived’ by apheresis before undergoing chemotherapy.

HOST LYMPHODEPLETION

The post-transplant setting is an ideal platform for adoptive immunotherapy strategies to capitalize on homeostatic T-cell proliferation,[98] in which naive T cells begin to proliferate and differentiate into memory-like T cells when total numbers of naive T cells are reduced below a certain threshold.[123,124] Host lymphodepletion may enhance the effectiveness of adoptively transferred T cells.[98] Homeostatic T-cell proliferation can result in the induction of auto-immunity,[125] providing a clue to improved anti-tumour strategies. T cells can undergo up to

seven rounds of cell division after being deprived of contact with APCs.[45,126] This homeostatic response of T cells is directed largely to self-antigens.[127] This hypothesis has been tested clinically in patients with metastatic melanoma refractory of conventional treatments.[62] In this study, a high rate of clinical disease regression was observed in patients who received a lymphodepleting conditioning regimen consisting of cyclophosphamide (60 mg/kg × 2 days) and fludarabine (25 mg/m^2 × 5 days) prior to adoptive transfer of T cells.

The authors have completed two Phase I trials in patients transplanted for haematological malignancies using autologous T cells stimulated by coculture with immunomagnetic beads to which anti-CD3 and anti-CD28 monoclonal antibodies had been conjugated. In the first trial, patients with relapsed or refractory non-Hodgkin's lymphoma were treated with CD34+-selected haematopoietic SCT followed by infusion of up to 1 × 10^{10} CD3+ autologous T cells at Day 14 after transplant.[128] Infusion of autologous costimulated T cells resulted in a rapid, dose-dependent reconstitution of lymphocyte counts. Importantly, the expanded cells were functionally superior to those obtained directly from the patients, as determined by interferon-gamma induction. Complete or partial responses were observed in 10 of the 16 patients infused. ALC recovery was compared with historical controls and found to be superior, but the clinical response was not compared.

In a similar Phase I/II trial, the authors examined the role of pre-transplant immunization and T-cell add-back in autologous transplantation for multiple myeloma.[78] All patients received two doses of Prevnar, the 7-valent pneumococcal conjugate vaccine (PCV), beginning 1 month after transplant. Half of the patients received an additional PCV vaccine 2 weeks prior to a steady-state leukapheresis. The harvested T cells were expanded in vitro using the beads described above. Patients received a standard, non-lymphocyte-depleted, autologous SCT after melphalan conditioning, and then received up to 1 × 10^{10} autologous, expanded CD3+ T cells either 14 days or 100 days after transplantation. As expected, early T-cell recovery was observed in both patient groups that received T-cell add-back on Day 14, while the groups that received add-back on Day 100 were significantly lymphopenic. The authors also found that only those individuals who received PCV-primed T cells early after transplant developed and maintained protective levels of anti-pneumococcal antibodies, as well as PCV-specific CD4 responses. Notably, T-cell responses to antigens not included in the vaccine were also improved in this group. These data demonstrated that combination immunotherapy consisting of a single early post-transplant infusion of antigen-primed, ex-vivo costimulated autologous T cells followed by post-transplant booster immunizations improved the severe immunodeficiency associated with high-dose chemotherapy, and led to clinically relevant immunity in adults within 1 month following transplantation. This pilot study provided a 'proof-of-concept' and allowed the authors to extend their studies using activated T cells and cancer vaccines. Based on these early results, a new clinical trial was designed utilizing the 'prime-boost' strategy of pre-T-cell harvest vaccination followed by post-transplant booster vaccines. In this Phase I/II trial, myeloma patients are immunized with a multipeptide vaccine composed of HLA-restricted peptides from human telomerase (!hTERT), survivin and cytomegalovirus. Survivin and hTERT are both overexpressed in a number of malignancies, including myeloma.[129,130] The vaccinations are followed by T-cell harvest, autologous SCT and infusion of ~5 × 10^{10} expanded, autologous T cells at Day +2 after transplantation. The patients go on to receive three additional peptide immunizations. Thus far, T-cell numeric recovery is significantly greater in comparison with the two previous trials. Studies are in progress to determine the mechanism of the enhanced lymphocytosis and response to the peptide vaccine.

SUMMARY

The prolonged immune dysfunction seen after haematopoietic SCT can have devastating consequences, including serious viral and bacterial infection, and tumour relapse secondary to

impaired immune surveillance. At the same time, the lymphopenia observed during this period may provide an optimal environment for the adoptive transfer of T cells capable of generating an anti-tumour response and repairing overall immunity. However, although adoptive T-cell therapy of rodent malignancies was first reported in 1955,[131] no forms of T-cell therapy have been approved by the US Food and Drug Administration after more than 60 years of research into adoptive immunity for tumours. Still, there is increasing optimism that the scientific barriers preventing clinically effective adoptive immunotherapy have been addressed. Advances in the understanding of T-cell biology and the tumour micro-environment have provided multiple novel adoptive transfer strategies that are now poised for translation into clinical trials. Finally, it is likely that adoptive immunotherapy will not be used alone, but rather in combination with other forms of immunotherapy and chemotherapy to maximize both passive and active immunity.

Practice points

- haematopoietic SCT recipients are at increased risk for infectious complications in the immediate and late post-transplant periods
- successful immunization of transplant recipients relies on immune reconstitution of both T and B cells
- adoptive T-cell therapy in the immediate post-transplant period enhances quantitative and functional immune recovery, and may allow vaccine introduction and promote tumour immunity
- the dose and scheduling of adoptive T-cell therapy in the post-transplant setting has yet to be optimized, so careful monitoring for adverse events is an essential component of this treatment modality

Research agenda

- while DLI has proven efficacy for the treatment of chronic myeloid leukaemia, autologous T-cell infusions should still be viewed as experimental, and only offered within the context of a clinical trial
- determine optimal timing and doses of adoptively transferred T cells in patients after SCT to augment the effects of host lymphodepletion

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