Artigo Especial / Special Article

Donor lymphocyte infusion in bone marrow transplantation therapy

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The dose escalation of chemo-radiotherapy that is achievable with stem cell transplantation is often insufficient to eradicate malignancy, and an associated immune-mediated graft-versus-malignancy effect may be equally important for many diseases. The most directly compelling evidence for its presence has been provided by the efficacy of donor lymphocyte infusions (DLI) in generating anti-tumor responses, particularly for relapsed chronic-phase CML. Response rates and durability appear lower with myeloma and AML/MDS, and minimal with ALL. There is relatively little data on indolent lymphoid malignancies. Issues that remain to be resolved include the precise nature of the effector cells and their target antigens, the best strategies for separating graft-versus-malignancy from graft-versus-host disease (GVHD) and their effect on the durability of responses, and the role of adjuvant chemotherapy/ cytokines. Similar issues surround routine combination with nonmyeloablative transplantation protocols and preliminary data suggests that GVHD may continue to provide a major obstacle.
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Introduction

Stem cell transplantation has allowed escalation of chemo-radiotherapy towards the limits imposed by organ toxicities other than the bone marrow, resulting in significant associated morbidity. Enhancing malignant cell kill by further manipulation of the conditioning regimen is therefore difficult and focus has shifted towards attempts to exploit graft-versus-tumor responses, including both the use of DLI and the development of nonmyeloablative conditioning regimens. Until recently most of the evidence for the existence of these responses in humans was indirect, including the apparently protective effect of acute or chronic GVHD against relapse, and the higher rate of relapse following syngeneic and T-cell depleted transplants (reviewed in [1]). This review presents data on recent insights into the situations in which DLI may be useful and into the attempts to dissociate its benefits from GVHD.

Disease-Specific Responses

Ten years of experience has confirmed that DLI achieves the highest response rates in chronic phase CML (70-80%). Patients treated in blast crisis have a lower probability of achieving remission (12-28% [2;3]). The majority of patients who enter remission have no detectable minimal residual disease when analysed for BCR-ABL transcripts by RT-PCR (4). Responses appear to be durable in most patients treated in cytogenetic relapse or chronic phase,

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although there are few data on long-term follow-up and interpretation has been difficult due to inconsistencies in the definition of remission (4, 5). Of the 44 patients achieving molecular remission in the study by Dazzi et al, 4 subsequently become positive for BCR-ABL transcripts (4). With a median follow-up after molecular remission of 29 months (range 5-89), the duration of molecular remission had already exceeded that following the initial transplant in 68% of patients. However, it should be noted that a relatively large proportion of these patients underwent a T-cell depleted transplant procedure and that results following T-cell replete transplants may differ. It is also not known whether limiting T-cell dose will compromise the durability of remission. All 4 of the relapsing patients reported by Dazzi et al had received an escalating dose DLI protocol compared to 48% of the total transplant cohort (the others receiving a bulk-dose regimen).

Responses following DLI have been observed in a minority of patients with AML and MDS (15-30%) and are rare in ALL (2, 3, 6). Combination with re-induction chemotherapy has had little impact on overall survival. A recent study of 44 ALL patients included 29 treated with both chemotherapy and DLI (6). Relapse occurred within 200 days in 3 of 4 patients receiving DLI as consolidation of remission induced by chemotherapy or immunosuppression-withdrawal, and in all 5 patients who entered remission after receiving DLI in the nadir after chemotherapy (n=25). Responses have therefore not been as durable as they have been for CML, although some patients with AML have survived for more than 2 years (3). The estimated survival after DLI for AML/MDS and ALL at 5 years has been reported at 13% and 0% respectively (3).

The response rate in myeloma is probably greater than in patients with acute leukaemia but less than in CML. Salama et al reported a response in 10 of 25 patients with myeloma (7). In 3 of these DLI was combined with chemotherapy, and 5 of the 7 treated with DLI alone required escalating doses of donor T-cells in order to produce a response. Patients receiving cell doses of at least 1 x 10^8 T-cells/kg were more likely to have anti-tumor responses and all responders developed GVHD. The durability of responses is currently unclear but some may be transient and extramedullary relapses are common. Of the patients reported by Salama et al 4 had responses lasting >1 year, 3 <1 year, and 3 had ongoing responses with <1 year follow-up.

Current results suggest that the use of DLI to treat relapsed myeloma following allogeneic SCT may be limited by short duration of response and significant GVHD, perhaps relating partly to the high T-cell doses required to effect these responses. Strategies that use DLI as prophylaxis to prevent relapse may be more successful than using DLI to treat relapse, but need to be addressed in clinical trials (8, 9).A recent study by Badros et al reported preliminary results in a group of refractory or poor-prognosis patients after allotransplantation using a nonmyeloablative conditioning regimen and DLI (8). Fourteen patients received donor lymphocyte infusions starting 21-42 days post transplantation in order to attain full donor chimerism or to eradicate residual disease.

One patient failed to engraft and 5 received chemotherapy in addition to DLI. Of the remaining 8 patients, 6 demonstrated disease response that was maintained in 4 from 120 - 490 days post transplant. These initial results are encouraging in such a heavily pre-treated group but need to be confirmed and expanded upon with larger patient numbers and with longer follow-up. A high incidence of GVHD consistently preceded the development of GVM and contributed significantly to morbidity and mortality.

There are relatively few data to assess the effect of DLI on indolent lymphoma and chronic lymphocytic leukaemia. Registry data suggest that there may be a significant graft-versus-tumor effect (1). Post transplant lymphoproliferative disorders (PTLD) have been successfully treated with DLI following allogeneic SCT (10). Efficacy depends upon the restimulation of autologous EBV-reactive memory T-cells, which are maintained at high frequencies in normal individuals. For this

reason relatively low cell doses are likely to be effective and the antitumor response can be generated without significant GVHD. Early detection of PTLD can be afforded by monitoring EBV DNA load, which is of particular importance in high-risk patients in view of the aggressive nature of these disorders (11). This type of response could potentially be beneficial in Hodgkin's disease (HD) where EBV DNA can be localised to the malignant cells in 20-40% of patients. Few allogeneic transplants are done for HD but since the majority of donors are EBV-seropositive it should be possible to generate EBV-reactive donor T-cell lines for use as immunotherapy (albeit to a distinct subset of EBV proteins including LMP 1 and LMP 2) (12).

A relatively small number of allogeneic SCT have been performed for solid tumors. However, the limited available evidence does support the possibility of graft-versus-tumor effects for breast cancer and renal adenocarcinoma (13, 14). Whether this is clinically significant remains to be established but the introduction of less toxic nonmyeloablative transplants may result in larger studies to address this issue.

Unrelated Donors

Similar results have been achieved in CML with both HLA-identical sibling and matched unrelated donors (MUD) (15, 16). Whilst there is little data to suggest substantially higher rates or severity of GVHD in the unrelated donor setting, it is possible that those at highest risk may have already developed GVHD and either died or be deemed unsuitable candidates for DLI (16). The implications of DLI on the unrelated donor also raise complex ethical and logistical issues.

Effector Cells and Target Antigens

Elucidation of the precise nature of the effector cells of GVL may help to identify strategies to separate GVL from GVHD. However, the effector cells of both remain incompletely defined. T-cell depletion is known to result in an increased incidence of relapse of CML, and it is likely that donor T-cells are the primary mediators of GVL. Both CD4+ and CD8+ cytotoxic antileukaemic T-cell lines and clones have been reported (reviewed in [17]). However, such cells may potentially also mediate GVHD by cross-reaction with MHC-specific targets (18). Natural killer (NK) cells may also have a role in the induction of GVL, particularly in the HLA mismatched setting (19).

Identification of the target antigens for GVL responses may allow manipulation of DLI for clinical benefit. Truly tumor-specific targets and associated target-specific effectors have proven hard to demonstrate outside the setting of PTLD. Novel fusion peptides associated with disease-specific translocations could provide targets and the BCR-ABL peptide expressed in CML is one potential candidate which has been demonstrated to be able to serve as a target for T-cell immunity [20;21]. However, these fusion peptides may only provide a targeting signal if expressed on the cell surface and in many cases it is difficult to demonstrate BCR-ABL expression on the surface of CML cells. A recent study from Boston confirmed the presence of a CD4+ T cell clone selected from a CML patient after donor lymphocyte infusion which recognized BCR-ABL breakpoint peptides but not tumor cells (22).

Lineage-specific chimerism analysis has demonstrated that donor lymphocytes not only eradicate the host leukaemia but also the host non-leukaemic T-cells, suggesting that the effector cells may not be leukaemia-specific but are more likely allospecific in many situations (23, 24). Haematopoietic-tissue-restricted minor histocompatibility antigens (mHags) might provide a graft-versus-haematopoietic system target (25, 26). Normal cellular constituents that are expressed on the cell surface could also act as potential target antigens if abnormally or over-expressed. Examples include WT1 and proteinase 3 in CML and some cases of AML (27, 28) and MUC1, hTERT and possibly HM1.24 in multiple myeloma (and many solid tumors) (29). These targets could potentially result in GVL activity overlapping with and not entirely separable from GVHD.

Separating GVL from GVHD

The efficacy of DLI and the ease of administration are balanced by the potential for significant toxicity. The major toxicities are secondary to marrow aplasia and GVHD, which may occur in up to 50% and 90% of responders respectively (1). Marrow aplasia is usually transient and less common when DLI is administered early in the course of relapse, although it may require haematopoietic stem cell rescue using either donor marrow or mobilised donor peripheral blood stem cells.

The association of GVL and GVHD remains a limiting factor in the application of DLI. Attempts have therefore been made to dissociate one from the other. These can broadly be divided into 2 approaches. Firstly, attempts to reduce the number of potentially harmful alloreactive T-cells, and secondly, attempts to limit their function (Table 1). Limiting T-cell number has been shown to result in GVL in the absence of GVHD in some patients with CML (23, 30). Both show a dose response effect. Thus it may be possible to induce GVL at a T-cell dose below the threshold necessary to cause GVHD. However, the dose of DLI required to induce a graft-versus-malignancy effect is likely to differ between malignancies and according to the tumor mass at the time of infusion. DLI depleted of CD8+ cells has been shown to be effective in re-inducing remission in patients with relapsed CML with a reduced incidence of GVHD. Although the number of patients treated was relatively small there was a suggestion that the number of CD4+ T cells infused might correlate with the incidence of GVHD (31, 32).

Inflammatory cytokines released at the time of transplantation appear to play a major role in the development of GVHD and the time interval between SCT and DLI may be an important variable in the propensity for its development. There are few data to address the problem of how soon DLI can be given after transplantation without a high risk of GVHD. As few as 1×10^5 T-cells/kg are capable of causing GVHD if given on the day of transplant, whilst many patients given 1×10^7 T-cells/kg beyond 12 months post transplantation do not develop clinical GVHD (23). Whether the same number of donor lymphocytes could be given without toxicity following T-replete SCT is currently not known. The limited experience following nonmyeloablative transplantation demonstrates high incidences of GVHD following early administration of relatively large doses of T-cells (8, 33, 34). Information on when it is relatively safe to give a defined number of unseparated or CD8+ depleted T-cells post-SCT would be particularly useful in patients who relapse within a few months of transplantation and in the planning of trials combining SCT with pre-emptive DLI to enhance GVL activity.

Numerous techniques have been described for the in vitro expansion of tumor-specific CTL, including co-culture with dendritic cells pulsed with tumor cell lysates or putative tumor related antigens/peptides, or with hybridomas generated by the fusion of tumor cells with dendritic cells (21, 29, 35). Alternatively, tissue-restricted mHags can be utilised (25, 26). In many cases such CTL can be shown to lyse tumor cells. The enrichment for tumor-specific or mHag-restricted cells may tip the balance towards GVL and away from GVHD. Infusion of T-cell clones selected on their ability to lyse appropriate targets could reduce the risk of GVHD further. The recent development of tetrameric HLA class I-peptide complexes allow isolation of CD8+ T-cells specifc for either mHags or tumor-related antigens such as proteinase 3 (36).

This has opened the possibility of selecting these cells either following in vitro culture or directly from vaccinated donors. Many questions remain unanswered about the application of this technology to lymphocyte infusions. It is unclear what effect these manipulations may have on cellular function, and the durability of responses based on a CD8+ T-cell infusion alone are unknown. The effect of the lack of CD4+ helper function on durability of CD8+ responses after infusion of CMV-specific CTL clones has been documented in a similar setting (37). There is also the potential of selective pressure resulting from a restricted target repertoire leading to tumors

developing strategies of immune evasion (38), although these may be less if mHags are targeted.

Depletion of potentially alloreactive T-cells on the basis of expression of activation-induced antigens such as CD25 and CD69 in a mixed lymphocyte culture (MLC) (39-41), induction of anergy to allospecific targets (42), or sublethal irradiation (43) may all reduce the potential for GVHD, but it will be important to establish whether these approaches also diminish GVL activity or the duration of such responses. Similarly, whilst the principle of an ability to control GVHD using donor T-cells transduced with a suicide gene has been proven (44), it is probable that this approach would also abrogate GVL activity. Evidence for an effect on leukaemia-free survival is lacking and the in vitro manipulations may have effects on cell function and T-cell subset composition that need to be further elucidated since there is some data to support a reduced capacity of such cells to induce both GVL and GVHD reactivity (44). The demonstration of the differential usage of Fas ligand and perforin cytotoxic pathways by cells mediating GVL and GVHD in a murine model provides another alternative approach (45). Specific blockade of one pathway may limit GVHD without interfering with GVL activity but similar data in humans are lacking.

It has been suggested that Type-2 polarisation of lymphocyte subset composition may be protective against GVHD. Such polarisation is seen following G-CSF mobilisation (46) and this might help to explain why peripheral blood SCT has not resulted in an increased incidence of acute GVHD despite far higher numbers of T-cells being transferred at the time of transplantation compared to bone marrow SCT (47). In vitro or in vivo use of Type-2 cytokines (IL-4, IL-11) may therefore protect against GVHD but again the effects on GVL remain to be elucidated in a clinical setting. The role of Tr1 cells in suppressing both Type 1 and Type 2 responses is another potential target for attempts to induce tolerance and reduce GVHD [48]. However, whether it will be possible to manipulate these responses in any way to separate from GVL from GVHD remains to be proven.

Nonmyeloablative Conditioning Protocols

Many of the same principles apply to these approaches as to myeloablative transplantation (the reader is referred elsewhere for excellent summaries [1;49;50]). Aggressive malignancies are likely to progress before clinical benefit can be realised and the approach is therefore best suited to those diseases in a minimal residual disease state which are likely to remain stable for several months or longer (in a similar way to DLI). Early hopes of very limited GVHD due to less release of inflammatory cytokines coupled with the possibility of stable mixed chimerism have not been entirely realised. One approach has been to increase immunosuppression further with the incorporation of ATG or CAMPATH (51).

These approaches may reduce GVHD, perhaps in some ways analogous to T-cell depletion prior to myeloablative transplantation, and further follow-up is required in order to document effects on disease relapse. A longer delay in immune reconstitution may necessitate combination with more cytoreductive agents in order to allow time for the development of graft-versus-malignancy effects and subsequent DLI may also be required. It is likely that a spectrum of conditioning protocols will emerge along with programmes of adjunctive donor lymphocyte infusions and the true impact of graft-versus-malignancy on these neoplasms may take several years to establish.

Conclusions

The presence of a therapeutically beneficial graft-versus-malignancy effect has been demonstrated in a number of diseases, most notably in relapsed chronic phase CML. However, attempts to apply this form of therapy are still limited by a number of factors. Aggressive disorders with rapid cell growth currently remain relatively unresponsive with limited response duration, and the role of both adjuvant chemotherapy and cytokines remain to be elucidated. Separation of the beneficial graft-versus-malignancy effect from the harmful GVHD effect also remains a priority. Graded T-cell dose

protocols, lymphocyte subset selection and attempts to temporally separate donor lymphocyte infusion from the time of transplant are likely to continue to be important approaches. Alternative approaches based on a growing understanding of the effector cells and their target antigens need to be further explored.

It is important to remain aware that whilst a number of these approaches have been demonstrated to limit GVHD, their ultimate impact on the development and maintenance of GVL responses has yet to be determined. Finally, the place, timing and cellular constitution of donor lymphocyte infusions in the rapidly expanding field of nonmyeloablative transplantation will need to resolved, ultimately in the setting of randomised trials.

***Table 1**. Current and potential strategies to separate GVL from GVHD*

Reduction in number of potentially harmful alloreactive cells:
Administration of graded T-cell doses (23, 30)
Depletion of T-cell subsets (31, 32)
Depletion based upon activation marker expression in MLC (39-41)
Enrichment for antigen-specific T-cells e.g. in vitro expansion of tumor-specific or lineage-restricted mHag-specific CTL, or MHC-peptide tetramer complex selection
Reduction in function of potentially harmful alloreactive cells:
Reduce exposure to inflammatory cytokines – delay time to prophylactic infusion
Induction of anergy by blockade of co-stimulatory pathways (42)
Transduction with suicide gene (44)
Alteration of Type-1/Type-2 T-cell balance – e.g. collect T-cells at time of stem cell mobilisation, cytokine administration, regulatory T-cells
Blockade of specific cytotoxic pathways preferentially used by GVHD mediators (45)
Sublethal irradiation (43)

Infusão de linfócitos de doador na terapia ao transplante de medula óssea

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Resumo

A quimioterapia seqüencial e transplante de células progenitoras, habitualmente são insuficientes para erradicar a neoplástica e uma associação do efeito enxerto contra o tumor imune mediado pode ser importante para o controle de muitas doenças uma evidencia deste efeito é proporcionado pela eficácia da infusão de lifócitos de doadores com a finalidade de gerar respostas anti-tumoral particularmente na leucemia mielóide crônica. A freqüência de respostas, durabilidade aparentaram ser pequenas no mieloma e na LM1/SMD e mínima na LLA. Existem de dos insuficientes nos linfomas indolentes.

Vários aspectos necessitam ser esclarecidos incluindo a natureza presença das células efetoras e os antígenos alvos, e as melhores estratégias para separar o enxerto-versus-tumor da doença enxerto contra o hospedeiro, e o seu efeito na durabilidade das respostas e o seu papel como adjuvante no tratamento.

Aspectos similares permeiam também os protocolos de transplante não mieloablativos em que os dados preliminares apontem como maior obstáculo à doença enxerto contra o hospedeiro.

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***Palavras-chaves:** Transplante alogênico, infusão de linfócitos de doador, enxerto contra o hospedeiro, enxerto contra o tumor, imunoterapia passiva.*

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