Management of acute graft-versus-host disease

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Summary

Acute graft-versus-host disease (GvHD) is a frequent complication of allogeneic haemopoietic stem cell transplantation (HSCT) and donor lymphocyte infusions (DLI). Its incidence and severity depends on several factors, such as prophylaxis method, donor/recipient matching, intensity of the conditioning regimen and composition of the graft. Significant progress has been made in recent years in understanding the pathogenesis of the disease, and some of these advances have been translated into clinical trials. First-line treatment of acute GvHD is based on corticosteroids, and produce sustained responses in 50–80% of patients depending on the initial severity. Non-responders are offered second-line therapy, with combinations of immunosuppressive agents, but 1-year survival is 30% in most large trials. New strategies explored include infusion of expanded mesenchymal stem cells (MSC), down regulation of antigen-presenting cells (APC) and suicide gene transduced T cells. Acute GvHD is complicated by severe immunodeficiency causing life-threatening infections. To date, GvHD has not been differentiated from the graft-versus-leukaemia effect. The present review will discuss some of these aspects.

Keywords: graft-versus-host disease, haemopoietic stem cell transplantation, donor lymphocyte infusion, immunosuppressive therapy, mesenchymal stem cells.

Do we understand the pathogenesis of GvHD?

Although the pathogenesis of graft-versus-host disease (GvHD) is not the aim of the present review, a few words are warranted: indeed some of the recent advances in understanding cellular and cytokine mechanisms leading to GvHD have been useful in the clinic. GvHD is thought to be caused by donor T cells reacting against host allo-antigens: this may well be the case, but the mechanism underlying such immune reaction are quite complex (Teshima & Ferrara, 2002a). The early work of Ferrara and coworkers showed that inflammatory cytokines play a crucial role in the initial, amplification and cytotoxic phases of the disease (reviewed in Ferrara, 2002). Unfortunately, it seems that inactivation of cytokines, such as tumour necrosis factor (TNF) is not sufficient per se, and some initial success has come at the expense of increased infectious complications (Marty et al, 2003). More recently, the presentation of target antigens by host antigen presenting cells (APC) has been shown to be crucial for the initiation and development of acute GvHD (Shlomchik et al, 1999): in an early experiment, Shlomchik showed that mice with APC that were unable to present class I-restricted peptides (grafted from β2 knock-out), but that expressed class I on target tissues, were unable to develop acute GvHD (Shlomchik et al, 1999). Therefore host APC participate in activating donor T cells to kill host cells and the age of host APC seems relevant (in keeping with the notion that older patients have more GvHD) (Ordemann et al, 2002). The presentation of host antigens by host APC can be further enhanced by host gamma/delta T cells, as recently shown in the mouse (Maeda et al, 2005). The interplay of APC, T cells and cytokines is such that, in a mouse transplant model – with mismatch only between donor T cells and host APC, but not between donor and host gut-donor T cells still efficiently killed host gut cells, suggesting that tissue destruction can be achieved with a by-stander effect (Teshima et al, 2002b). Finally, attention has also been given to cellular interplay leading to tolerance: Two reports merit mentioning. Mc Donald et al (2005) dealt with cytokine-expanded myeloid precursors regulating APC and promoting tolerance through interleukin (IL)-10-producing regulatory T cells. Lan et al (2001) looked directly at regulatory T cells (TREGS): mice given lymphoid radiation and antithymocyte globulin (ATG) were depleted of most T cells, but not of NK 1.1 T cells, and became resistant to GvHD. The Stanford group has brought this in to the clinic: they have shown that the combination of total lymphoid radiation (TLI) and ATG depletes host T cells with the exception of CD4\(^+\)CD25\(^+\) TREGS: when allografted these patients showed little or no GvHD, suggesting a regulatory role of host TREGS on donor incoming alloreactive T cells (Lowsky et al, 2005).

Do we understand the pathogenesis of GvHD?

Not fully: however, we have become aware of the complexity of the disease, and we have learned to take advantage of some developments in the lab. Further studies in the animal model...
Can acute GvHD be accurately graded?

The original grading system for acute GvHD was proposed by Glucksberg et al. (1974) and identified five categories of patients (grades 0, I, II, III, IV). It is known as the Glucksberg-Seattle criteria (GSC) and it is based on the degree of skin, liver and gut involvement (skin rash, total serum bilirubin and diarrhoea volume), together with a subjective assessment of clinical status. The GSC have been widely used for 30 years and correlate well with transplant-related mortality (TRM): a recent analysis of 4174 human leucocyte antigen (HLA)-identical sibling transplants for chronic myeloid leukaemia (CML) in first chronic phase showed that early and long-term outcome is influenced by severity of acute GvHD as identified by the classic GSC (Gratwohl et al., 2002). Indeed at 3 years survival was 74%, 74%, 64%, 37% and 10% for patients with acute GvHD grades 0, I, II, III and IV respectively. In 1997, the International Bone Marrow Transplant Registry (IBMTR) designed a staging system from a large data set of adult patients receiving an HLA identical sibling BMT (Rowlings et al., 1997): the IBMTR Severity Index regroups patients into five categories (0, A, B, C, D) based on differences in TRM with a significance level of 0.05. Despite good correlation of the IBMTR index with outcome, the classic GSC is still used in most centres. Recently, we have shown that the GSC can be further implemented by adding platelet counts on day+50: patients with grade II acute GvHD and a platelet count of less than $5 \times 10^9/l$ had significantly higher TRM when compared to patients with grade II GvHD and platelets $\geq 50 \times 10^9/l$ (Dominietto et al., 2001).

Can we score GvHD?

Not well enough: however the classic GSC is still here after three decades, suggesting it is clinically useful. The use of laboratory values, such as platelet counts and cholinesterase, or other clinical parameters (Lee et al., 2005) may further improve our ability to predict the outcome of patient undergoing an allogeneic transplant, and perhaps to modify treatment accordingly.

Can GvHD be prevented?

HLA matching

HLA matching between donor and recipient is one of the most powerful predictors of GvHD, together with other factors such as age, donor/recipient sex match. Other supportive measures, such as a sterile environment have been shown to protect against GvHD both in animals and humans. Therefore using a young-HLA matched, male donor, in a protected sterile environment, is a very effective way of having little GvHD. However, HLA-matched related donors are less common, at least in Europe and the USA, and there is greater use of unrelated donors or related HLA-mismatched donors. Thus the question really is: can GvHD be prevented in less favourable situations?

T cell depletion ex-vivo

Removal of T cells from the stem cell suspension, referred to as T cell depletion (TCD) ex-vivo, was very popular in the 1980s, but its use has declined over the past decade: this is because survival, disease-free survival and transplant mortality were not reduced, in the setting of HLA-matched grafts, when compared with conventional unmanipulated transplants (Marmont et al., 1991). One situation in which ex vivo-TCD is essential, is 3 loci mismatched transplants: in this setting T cell depletion must be thorough and one should not infuse more than $5 \times 10^4$ CD3+ cells/kg of recipients weight, as shown by Aversa et al. (1998). TCD should be performed in a centre with a dedicated programme, where different forms of T cell removal can be explored, including physical and immunological TCD (Martin & Kernan, 1997; Aversa et al., 1998).

T cell depletion in vivo

Treatment of the patient with T cell antibodies before the transplant, in vivo-TCD, has a double target: it reduces the host immune response, favouring engraftment, and downregulates donor T cells, because the antibody is still in circulation at the time of transplant, and thus prevents GvHD (Hows et al., 1993; Holler et al., 1998; Baermann et al., 1999; Zander et al., 1999; Byrne et al., 2000; Finke et al., 2000). In vivo-TCD is indicated in programmes involving alternative donor transplants, and is used in many, but not all centres performing unrelated donor grafts (Hows et al., 1993; Hansen et al., 1998; Holler et al., 1998; Baermann et al., 1999; Zander et al., 1999; Byrne et al., 2000; Finke et al., 2000). In a recent randomised trial, we showed that rabbit anti thymocyte globulin (ATG) significantly reduced the risk of grade III–IV acute GvHD in unrelated donor transplants (Bacigalupo et al., 2001a). However, TRM and survival were unchanged due to a higher risk of lethal infections in the ATG 15 mg/kg arm (Bacigalupo et al., 2001a). An update of the same study showed that ATG provided significant protection against acute and chronic GvHD, shortened time to terminate immunosuppression and improved quality of life (Bacigalupo et al., 2006). Whether different schedules of administration of ATG or different agents may further improve results remains to be determined.

Expanding regulatory T cells (TREGS)

CD4+CD25+ immunoregulatory T cells (TREGS) can be administered to inhibit GvHD while preserving graft-versus-leukaemia activity after allogeneic bone marrow transplantation in mice. Preclinical studies suggest that it is
necessary to infuse as many TREGS as conventional donor T cells to achieve a clinical effect on GVHD (Trenado et al., 2006). How do we translate this to the clinic? The Stanford group has designed a transplant programme consisting of fractionated TLI (800 rads delivered in 2 weeks, 80 rads/d $\times 5 \times 2$) and ATG (5 d) followed by allogenic unmanipulated peripheral blood transplants (Lowsky et al., 2005), resulting in little or no acute GvHD. A recent update of that study at the 2006 meeting of the European Group for Blood and Marrow Transplantation (EBMT) has confirmed these results of 64 patients, half of whom received grafts from unrelated donors (R. Lowski, unpublished observations).

**Post-transplant immunosuppressive therapy**

This therapy is still the conventional form of GvHD prevention both in HLA-identical as well as unrelated-donor transplants. It is usually based on the combination of cyclosporin A (CsA) and short course methotrexate (MTX) on days+1, +3, +6, +11 (Storb et al., 1986; Zikos et al., 1998). The CsA dose used in the first 10 d post-transplant may have a significant impact on leukaemia control: in two prospective randomised trials, both in children and in adults, low-dose CsA (1 mg/kg) was shown to protect patients from leukaemia relapse when compared with higher doses of CsA (3 mg/kg or 5 mg/kg) (Bacigalupo et al., 1991; Locatelli et al., 2000). This was recently confirmed at a 10-year follow up (Bacigalupo et al., 2001b) and should be kept in mind especially when grafting patients at high risk of relapse. Recently tacrolimus (FK506), a calcineurin inhibitor, has been introduced in the prophylaxis of GvHD: 180 patients grafted from a matched unrelated donor were randomised to receive CsA+MTX or FK506+MTX (Nash et al., 2000). Acute GvHD II-IV was significantly lower (51%) in FK506-treated patients when compared with the CsA patients (70%) ($P = 0.0002$), but this did not translate in a lower risk of chronic GvHD. The adverse events, in particular nephrotoxicity, infections or leukaemia relapses were not significantly different (Nash et al., 2000). There was also no difference in survival. Therefore, both CsA+MTX and FK506+MTX combinations offer some protection for GvHD and have significantly reduced the risk of severe GvHD when compared to single agent prophylaxis (MTX or CyA alone). Mycophenolate mofetil (MMF) has been successfully introduced for GvHD prevention, and may substitute MTX in the standard CsA combination, mainly because of less mucositis and overall good tolerance (Basara et al., 2000; Nash et al., 2005; Neumann et al., 2005).

**Mesenchymal stem cells**

Mesenchymal stem cell (MSC) are pluripotent stem cells capable of generating osteoblasts, myoblasts, chondroblasts, tenoblasts, adipocytes and stromal cells (Pittenger et al., 1999). There have been several reports on the immunosuppressive effect of MSC, both in vitro (Klyushnenkova et al., 1998; Tse et al., 2000). and in vivo (Bartholomew et al., 2002). The co-infusion of a large number of osteoblasts together with hematopoietic stem cells, in a mismatched mouse model, resulted in successful engraftment and immune reconstitution (El-Badri et al., 1998), whereas control animals died of GvHD or rejection. A recent trial has been completed in 40 patients with haematological malignancies, using expanded MSC from an HLA-identical sibling, and co-infusing these cells with a conventional unmanipulated bone marrow (BM) or peripheral blood (PB) transplant: the infusion of MSC was safe, and this was the primary end point of the study (El-Badri et al., 1998); in addition, hematopoietic reconstitution was prompt. However, the study failed to show a significant reduction in acute or chronic GvHD when these patients were compared with a matched pair cohort of CIBMTR patients (El-Badri et al., 1998). MSC are now being co-transplanted in different settings, such as cord blood or unrelated marrow grafts. One has to note that the number of MSC or osteoblasts given to humans by at least 3 logs (Lazarus et al., 2005): if we want to achieve what has been shown in the experimental models, perhaps we will need to escalate the number of MSC we infuse.

**Inactivation of antigen presenting cells**

Depleting host APC before the conditioning regimen may reduce GvHD: in keeping with this observation, patients receiving extracorporeal photopheresis (ECP) before the conditioning regimen have a low incidence of GvHD (Chan et al., 2001), because ECP may downregulate host APC (Gorgun et al., 2001). It is also understood that broad specificity T cell antibodies (ATG and CAMPATH), commonly used in allotransplants, significantly deplete host APC cells. An other way of killing APC is by using natural killer (NK) cells. Ruggeri et al. (2001) showed that elimination of host APC in the mouse, using donor incompatible NK cells, made the recipients resistant to GvHD (they could be infused with large numbers of mismatched T cells, without GvHD). Thus expanding donor NK cells may be one way of reducing GvHD, and some centres have started to investigate whether donor NK cells can be expanded and co-infused to reduce GvHD, and possibly also to kill tumour cells (Aversa et al., 1998). Indeed, in the best possible donor-recipient combination, donor NK cells would reduce GvHD (by killing host APC), reduce rejection (by killing host T cells) and reduce relapse (by killing leukaemia cells). Whether we will be able to use NK cells so cleverly remains to be determined.

**Reduced-intensity conditioning regimens**

Reduced-intensity conditioning (RIC) regimens were introduced in the late 1970s when busulfan 8 mg/kg was used instead of the conventional 16 mg/kg, in children with inborn errors (Hobbs et al., 1981). These regimens are usually associated with less severe (or delayed) acute GvHD, because
of the persistence of host cells (mixed chimaeras), which counteract GvHD (Mielcarek & Storb, 2005), although early GvHD may still occur (Mielcarek et al, 2005). There may also be a lower level of inflammatory cytokines. RIC regimens based on low-dose TBI (2 Gy) (Storb et al, 1999), low-dose busulfan (Slavin et al, 1998), low-dose thiopeta (Raiola et al, 2000) low-dose melphalan (Kottharidis et al, 2000) or cyclophosphamide and fludarabine (Khouri et al, 1998) are being used widely in elderly patients up to the age of 70 years, and the overall risk of GvHD is probably less than what would be seen in patients of the same age with conventional intensity regimens. Acute GvHD still remains a major obstacle after RIC HSCT, occurring in 15% of patients in its severe form (grade III–IV), while extensive chronic GvHD is diagnosed in 50% of all patients (Bacigalupo, 2002). Several questions need to be answered in this context: timing and intensity of in vivo immunosuppression, timing of CsA discontinuation, timing of DLL and use of T-cell antibodies. Some regimens include anti-T cell antibodies, such as alentuzumab (CAPATH) (Kottaridis et al, 2000) or ATG (Slavin et al, 1998). It is interesting that Russell and coworkers have taken a different approach: they used ablative doses of intravenous busulfan combined with fludarabine and tried to maximise GvHD prevention with rabbit ATG in the conditioning regimen and CsA MTX for postgraft immunosuppression (Russell et al, 2002). The programme, designated FLU-BUP, was given up to the age of 65 years, for patients with haematological malignancies, and yields a TRM of 4% in HLA-identical siblings and 20% in unrelated transplants.

Anti-IL2 anti-TNF antibodies

Other monoclonal antibodies (mAbs) that interact with IL2 or TNF have been tested in the clinical setting. Anti-CD25 mAb seemed to delay the occurrence of GvHD; in a randomised trial the administration of a CD25 mAb (in addition to CsA+MTX) appeared to decrease leukaemia-free survival, in comparison to conventional GvHD prophylaxis (Blaise et al, 1995). A humanised CD25 mAb, assessed in a double-blind, placebo-controlled randomised study, involving a total of 210 patients, failed to prevent GvHD or improve the outcome of unrelated HSCT recipients (Anasetti et al, 1995). A mAb neutralising TNF-α has also been tested in 21 patients as GvHD prophylaxis: in a prospective trial Holler et al (2002) showed that GvHD could be delayed (by 10 d), although the overall grading was similar to controls.

Can GvHD be prevented?

The answer is clearly yes, and there has been a progressive decline in the severe form of acute GvHD (III–IV) in the last decade (Bacigalupo et al, 2004). Whether reduction of GvHD produces improved survival, is a more difficult question. However, this should not discourage further studies on GvHD prevention, because if survival is comparable, morbidity is clearly lower if patients do not experience acute or chronic GvHD.

Can acute GvHD be treated?

First-line treatment

Corticosteroids Corticosteroids are used as first-line therapy: in a study of 443 patients who received prednisone 60 mg/m² for 14 d as first-line therapy, followed by an 8-week taper, an overall improvement was observed in 55% of the patients with durable (≥228 days) complete responses in 35% (Blazar, 2002). The probability of survival at 1 year after initiation of therapy was 55%; favourable predictors of survival were younger patient age, HLA-identical sibling donor and GvHD prophylaxis other than ex-vivo T cell depletion (Blazar, 2002). The authors of this important study concluded that steroids provide an active but inadequate therapy for acute GvHD, especially in patients with severe GvHD, and that more effective prophylaxis for mismatched and unrelated donor transplants is needed. Unfortunately, there is no evidence that more aggressive first-line therapy is beneficial: the Italian Group for Marrow Transplantation (GITMO) could not show an advantage of high-dose steroids (10 mg/kg of 6-MPred) over conventional 2 mg/kg MPred, with a transplant mortality at 1 year of 30% in both groups (Van Lint et al, 1998). A randomised study comparing steroids + ATG versus steroids alone came to the same conclusion (Cragg et al, 2000). Two additional observations were made in the GITMO study: (i) despite the very early day of randomization (median day +12 from transplant), high dose MPred did not prevent progression towards grade III–IV GvHD and (ii) responders to 5 d of 6MPred 2 mg/kg had a significantly lower TRM (16%) when compared with non-responders (46%) (Van Lint et al, 1998). We have confirmed this result in a more recent study (Van Lint et al, 2006).

Thus, primary treatment of acute GvHD should be prednisone or 6MPred 2 mg/kg/d for 5 d (Fig 1): responsive patients should taper steroid therapy. An IBMTR survey confirmed that a 5-d course is sufficient to identify steroid-refractory acute GvHD (Hsu et al, 2001). There may be less agreement on criteria to define response on day+5 or day+7: it is unlikely that patients will be free of GvHD. The most common situation will be reduction of clinical signs of acute GvHD, which may allow reduction of the 6MPred dose (Fig 1): indeed in two consecutive studies we found it very useful to identify day+5 responders as those patients who were considered eligible for MPred dose tapering (Cragg et al, 2000; Van Lint et al, 2006). The probability of response will be higher for patients who have limited acute GvHD on day 0 of treatment (Van Lint et al, 2006). Patients not responding on day+5 or day+7 could not reduce their dose of steroids and were eligible for second-line treatment (Fig 1). Transplant mortality is higher (as expected) in non-responders as compared to day+5 responders, and this indicates that
Second-line treatment

A large number of studies have been performed for patients not responding to 2 mg/kg of MPred (Fig 1). Most of these were phase II uncontrolled studies, showing some degree of response with the use of a large variety of agents. Very few were prospective randomised trials, and these have failed to show any advantage of experimental treatment over standard steroid therapy.

Anti-thymocyte globulin (ATG) Anti-thymocyte globulin is one treatment option for steroid-refractory patients, and some encouraging results have come from phase II trials (Roy et al, 1992; Aschan, 1994), especially when ATG was given early after the diagnosis of GvHD (MacMillan et al, 2002). However, we are lacking evidence that ATG improves survival. In a recent prospective randomised trial the Italian group GITMO has been unable to confirm that ATG is beneficial as a second-line therapy of steroid-refractory acute GvHD (Van Lint et al, 2006): 61 patients not responding to 5 d of MPred 2 mg/kg, were randomised to receive MPred 5 mg/kg/d for 10 d, alone (n = 34) or in combination with rabbit ATG (n = 27). The two groups were balanced for clinical and GvHD characteristic. One-month after randomisation 26% had a complete response, 25% a partial response, 33% stable GvHD, 10% worsened and 8% had died: there was no significant difference in response, TRM and survival between the non-ATG and ATG group (Van Lint et al, 2006). Five-year actuarial survival was 36% and 34% for controls and ATG patients.

Therefore, although ATG can induce a significant response in GvHD patients, survival is unchanged when compared with patients not receiving ATG; clinical response of GvHD does not mean improved survival, and responders may still die of infections and other complications, as suggested in a recent review (Antin et al, 2004).

Interleukin 2 receptor antibodies

There are several monoclonal antibodies to IL2 (reviewed in Antin et al (2004): denileukin difitox (Ontac), inolinomab (Leukotac), Basiliximab (Simulect), Daclizumab (zenapax). They have all shown efficacy in steroid-resistant acute GvHD (Antin et al, 2004). Nevertheless, infections remain a serious problem: is this due to the fact that patients come to monoclonal antibody therapy after failing several lines of treatment? Perhaps early administration of IL2R antibodies would prove more effective. A large prospective randomised trial is ongoing within the National Marrow Donor Program, testing, among other agents, an IL2 receptor antibody.

Anti-CD147 monoclonal antibody

Deeg et al (2001) have reported a pilot study on the use of anti-CD147 (a neurothelin member of the immunoglobulin superfamily which is upregulated on activated T and B cells): 27 patients with GvHD entered this study and 51% were considered as responders, including 25% complete responses. Survival at 6 months was 44% (Deeg et al, 2001).

TNF antibodies

Tumour necrosis factor is of one the inflammatory cytokines mediating cellular cytotoxicity. TNF can be downregulated by steroids, pentoxifilline, transforming-growth factor beta (TGFβ) and IL4. Antibodies to TNF (infliximab) or to the TNF receptor (etanercept) have been developed and used both in first- and second-line treatment of acute GvHD (Marty et al, 2003; Couriel et al, 2004; Uberti et al, 2005). Responses are seen, some patients clear their symptoms rapidly, but infections remain an issue (Marty et al, 2003).

Other agents

Other agents, such as MMF are being tested with some success: MMF has a good efficacy profile, is well tolerated in general and may prove useful in sparing steroids and steroid-associated complications (Antin et al, 2004).

Pre-emptive treatment

We have previously shown that patients at high risk of GvHD and TRM can be identified on day +7 following an allogeneic
bone marrow transplant (BMT), based on serum bilirubin and blood urea nitrogen levels (Bacigalupo et al, 1999): we have recently revised the scoring system with the inclusion of day+7 cholinesterase, gamma-glutamyltransferase, total protein together with cell dose and donor type (Sormani et al, 2003). One possible approach to reduce the risk of GvHD and TRM, is pre-emptive treatment before the disease develops. In a pilot study, we tested the feasibility of this approach in patients undergoing an alternative donor HSCT: the risk of severe GvHD and the actuarial 1 year TRM was reduced in the ATG treated patients (Bacigalupo et al, 2001c). This is in keeping with a previous randomised trial, published some years ago, showing that early administration of ATG after an HLA-identical sibling transplant, could significantly reduce the risk of acute GvHD (Ramsay et al, 1982). Whether this will translate in survival advantage is being now tested in a prospective multicenter trial by GITMO.

**Cellular therapy of GvHD**

This section includes the use of MSC, ECP directed against APC, and suicide gene transduced T cells

**Suicide gene transduction of T cells** This approach is based on transducing T cells with a gene, such as herpes simplex virus thymidine kinase (HSV-TK) which renders them susceptible to killing by ganciclovir, provided the cell is also dividing. The T cells are infused into the host and when GvHD develops, ganciclovir is given, killing only transduced T cells. This system requires efficient gene transduction and the ability to select transduced cells. It has been implemented in humans and animals models with some positive results, although anecdotical (Bonini et al, 1997). The model is based on the hypothesis, that established GvHD can be turned off by killing alloreactive T cells, although there is scanty evidence that this can be achieved: the activation system is very complex, and by the time it is established there are many cell types (both of donor and recipient origin) and many cytokines involved, often with opposite actions. During induction phase the administration of IL12 inhibits GvHD (Sykes et al, 1995). If, in the same mouse model, IL12 was given later after transplant, there was enhancement of GvHD by induction of host-derived interferon-γ (IFNγ) (Sykes et al, 1999). T cells from IFNγ knock-out mice, surprisingly, caused a more virulent GvHD, suggesting a protective effect of IFNγ (Murphy et al, 1998). In a different mouse P↑F1 model, the lack of IFNγ T cells delayed GvHD (Minasi et al, 1993). These results outline the complex pathogenesis of GvHD and the multiple effectors involved, which may vary according to the animal model chosen. If this is the case, can GvHD be turned off by killing T cells, and if so, which T cells need to be killed? Although suicide gene transduction was reported almost 10 years ago, and despite some pilot phase I/II studies, it has not been used in the clinic as a standard approach for acute GvHD prevention and treatment: it faces numerous problems, including logistic, technical and conceptual. For the time being, it remains an interesting investigational tool.

**Mesenchymal stem cells** There has been great interest in the use of MSC for treatment of acute GvHD in the past few years (Ringden et al, 2006): at the EBMT meeting 2006 we presented a cooperative report on patients treated in Huddinge, Genova and Pavia, and we further updated this report at the 2006 ASH meeting (Le Blanc et al, 2006): 40 patients with grades III–IV acute GvHD receiving MSC were evaluated. The median MSC dose was 1×10⁶ (range 0–4–9) 10⁶ cells/kg body weight of the recipient. No side-effects were seen after MSC infusions. Nineteen patients received one dose, 19 patients received two doses, two patients received three and five doses, respectively. MSC donors were HLA-identical siblings in five cases, haploidentical in 19 cases and third-party HLA-mismatched in 41 cases. Among the 40 patients treated for severe acute GvHD, 19 had complete responses, nine showed improvement, seven patients did not respond, four had stable disease and one patient was not evaluated due to short follow-up. Twenty-one patients were alive between 6 weeks up to 3-5 years after transplantation, Nine of whom had extensive chronic GvHD. These results suggest that immunomodulatory and tissue repairing effects of MSC should be further explored as treatment of severe acute GvHD in prospective randomised trials, especially as there have been negative reports in the experimental model (Sudres et al, 2006).

**Extracorporeal photopheresis**

Extracorporeal photopheresis has been mostly used in patients with chronic GvHD, and significant responses have been seen in a proportion of patients (Couriel et al, 2006). A randomised study has been completed in chronic GvHD patients, and should be available for definitive analysis this year (Greinix et al, 2006a). Recent reports have been published, of ECP used for acute GvHD: the procedure is invasive, requires a dedicated team at the Blood Bank and responses are not seen before 12 weeks, therefore requiring long-term supportive care (Garban et al, 2005; Greinix et al, 2006b). However, given the current poor results with second-line therapy, waiting 12 weeks for a response would not be the greatest of problems. Greinix et al (2006b) reported an 82% complete response rate for patients with severe skin acute GvHD treated with ECP and steroids, 61% for gut involvement and 61% for liver involvement. A randomised trial comparing prednisone + ECP versus prednisone alone has been activated this year.

**Can GvHD be treated?**

The answer is a cautious ‘Yes’. Most events occur very early in the course of the transplant. First-line therapy with conventional dose steroids (1–2 mg/kg/d) can be successfully given to over half of affected patients. Response depends on the severity of GvHD and organ involvement. If patients respond, their
transplant mortality is relatively low; if they don’t respond to first-line treatment, their transplant mortality is high (possibly exceeding 60%). Mortality after second-line therapy has not been reduced in the last three decades: patients do respond to second-line therapy and GvHD severity is diminished, but the patients may still succumb to infectious complications. New agents, antibodies or intensified immunosuppression have not changed GvHD-related mortality, cellular therapy holds promise. The important take-home message is: whatever you decide to do, please enter your patients in to a clinical trial (Bolanos-Meade & Vogelsang, 2005): this is the only way we can expect to make progress in the near future.

Can we improve immune-reconstitution?

Immune reconstitution remains a significant problem following allogeneic stem cell transplantation and several studies have analysed this. The number of NK cells, as monitored by CD56 or CD16 expression on peripheral blood lymphocytes, rose rapidly after transplant, and returned quickly to the normal range while CD8+ cells frequently remained high (Dokhelar et al, 1981). There is a strong imbalance of helper/suppressor cells, first described many years ago (Bacigalupo et al, 1981), which still needs to be fully understood. It may be associated with immune reconstitution or with alloreactivity (Noel et al, 1978; Paulin et al, 1987; Barrett et al, 2003): the former seems more probable, since the CD4/CD8 ratio is not a good indicator of acute GvHD. Other factors associated with poor immune recovery also predict GvHD: older age (Seddik et al, 1984), unrelated donors (Small et al, 1999) and of course steroid therapy given to treat GvHD (Douek et al, 2000). The thymus plays an important role in reconstitution of T-cell immunity and increasing patient age has an adverse effect on the regeneration of naive CD4+ T cells, probably due to age-related thymic involution: these observations were confirmed by TCR rearrangement excision circle (TREC) assay to measure thymic output (Douek et al, 1998, 2000; ). Attempts to boost immune reconstitution by IL7 or IL7-engeneered cells have been reported (Li et al, 2006), but we have not yet seen these studies translated in the clinic. Recently, keratinocyte growth factor (KGF) has been shown to promote immune reconstitution after HSCT in the animal model (Min et al, 2002): trials using KGF are underway in clinical allograft programs.

Immune reconstitution is inversely correlated with the severity of GvHD: the more severe the disease the worse the immune recovery (Sale et al, 1992). The situation is worsened by treatment of GvHD with steroids, antibodies and other suppressive manoeuvres. Attempts to manipulate immune recovery with cytokines have failed so far, although IL7 holds promise. Infections are a frequent and often lethal complication: they include viral, fungal and bacterial infections in patients with GvHD. Discussing these complications is outside the scope of the present review, however, monitoring and early treatment of infections should be a major part of standard protocols or prospective trials. This is also relevant because of the many old and new agents available to treat cytomegalovirus (CMV), Epstein–Barr virus (EBV), aspergillus and vancomycin-resistant enterococci.

We can not leave this section without mentioning the elegant studies, pursued by several groups, in expanding antigen-specific T cells (Peggs et al, 2001; Rauser et al, 2004; Perruccio et al, 2005; Bollard et al, 2006; Riddell et al, 2006): immunity to CMV, EBV and aspergillus has been successfully transferred with expanded antigen-specific T cells, with no GvHD, and with clearance of the infection. A very powerful proof-of-principle indeed. Thus the technology is now established, but is the cost and the laboratory resources affordable for most transplant centres? Possibly not, but developing this technology should advance our ability to manipulate the immune system, and this is important for the management of transplants, and possibly also for the treatment of tumours.

Is acute GVHD influenced by stem cell source?

What about the three stem cell sources: PB, BM, cord blood (CB). They are listed here in the order of current ‘preference’, because numbers do represent what happens every day in our centres. The last EBMT survey told us that allogeneic PB is the preferred source, followed by BM and CB. The influence on acute and chronic GvHD is probably also falls in the same order; PB, BM, CB.

There are four large international randomised studies that used BM or PB as the source of stem cells (Blaise et al, 2000; Bensinger et al, 2001; Couban et al, 2002; Schmitz et al, 2002).

Acute GvHD grade III–IV was similar in three studies, and significantly increased in PB recipients in one study (Schmitz et al, 2002). It is therefore reasonable to say that acute GvHD was rather comparable in patients receiving allogeneic BM or PB cells, although chronic GvHD was significantly increased in almost all studies. Three recent publications have set the stage for HLA-identical sibling CB transplants (Rocha et al, 2000) and for unrelated CB transplants in children (Rocha et al, 2001) and in adults (Laughlin et al, 2001). Common to these three publications is the low risk of acute and chronic GvHD for patients receiving CB transplants: in the HLA-identical study, the risk of acute GvHD was 0.41 for CB when compared with BM ($P = 0.001$) and for chronic GvHD the risk was 0.35 ($P = 0.02$).

But, perhaps comparing unmanipulated PB, BM and CB is not the right question at present: efforts should be made to optimise programs involving each of these stem cell sources: we will then find patients who might benefit from the different impact these stem cell sources have on GvHD and outcome.

Conclusions

Treatment of established GvHD is complex and has not made major advances in the last decade. Possibly we treat our patients too late, when tissue damage has already taken place.
and cytokine production by activated donor T cells and autologous macrophages can proceed undisturbed. It would seem that acute GvHD is, to a certain extent, self-programmed, since very early treatment with high dose corticosteroids and/or ATG does not modify the natural course of the disease. Over the last three decades we have considerably reduced the risk of acute GvHD and understood how to prevent it by modifying the transplant program and the stem cell source: we now need to improve our ability to predict GvHD and develop new strategies of early treatment.

References


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