Management of acute graft versus host disease (GvHD)

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Graft versus host disease (GvHD) is a frequent complication of allogeneic hematopoietic stem cell transplantation (HSCT), and of donor lymphocyte infusions (DLI): the acute form occurs within 100 days from HSCT or DLI, the chronic form beyond day +100. GvHD should be prevented rather than treated. There are several ways to prevent GvHD: remove donor T cells from the transplant (ex vivo T-cell depletion), administer T-cell antibodies to the patient (in vivo T-cell depletion), administer immunosuppressive drugs such as methotrexate, cyclosporin, tacrolimus, and mycophenolate (post-transplant immunosuppression). New strategies of GvHD prophylaxis include the infusion of expanded mesenchymal stem cells and downregulation of host antigen-presenting cells. First-line treatment of established GvHD is based on low-dose corticosteroids (0.5–2 mg/kg). Second-line therapy for steroid refractory GvHD is unsatisfactory. The early administration of T-cell antibodies and/or TNF antibodies and TNF soluble receptor may be successful in some cases, but they do not seem to fulfill the expectations. High-dose chemotherapy has not been explored thoroughly. Acute GvHD is complicated by infections that cause significant morbidity and mortality: prophylaxis, early diagnosis and treatment of infections are an integral part of GvHD management. We have considerably reduced the risk of acute GvHD over the past three decades: we need to further improve these results, with the final goal of dissecting, if possible, the graft versus host from the graft versus tumor effect.


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Grading of acute GvHD

The original grading system for acute graft versus host disease (GvHD) was proposed by Glucksberg et al.1 in 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 diarrhea volume), together with a subjective assessment of clinical status. The Glucksberg criteria have been widely used for 30 years and correlate well with transplant-related mortality (TRM): a recent analysis of 4174 HLA identical sibling transplants for chronic myeloid leukemia (CML) in the first chronic phase has shown that early and long-term outcome is influenced by the severity of acute GvHD as identified by the classic GSC.2 Indeed at 3 years survival was, respectively, 74, 74, 64, 37 and 10% for patients with acute GvHD grades 0, I, II, III, 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:3 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 centers. Recently, we have shown that the GSC scoring system can be further implemented by adding platelet counts on day +50: patients with grade II acute GvHD and a platelet count of less than 500 × 10^9/l had significantly higher TRM as compared to patients with grade II GvHD and platelets ≥50 × 10^9/l.4 The use of laboratory values, such as platelet counts and cholinesterase, may further improve our ability to predict the outcome of patients undergoing an allogeneic transplant, and perhaps to modify treatment accordingly.

GvHD prophylaxis

T-cell depletion ex vivo (TCD)

Removal of T cells from the stem cell suspension, referred to as 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 are not reduced, in the setting of HLA matched grafts, when compared to conventional unmanipulated transplants.5 One situation in which ex vivo-TCD is essential is three loci mismatched transplants: in this setting T-cell depletion must be thorough and one should not infuse more than 5x10^4 CD3+ cells/kg of recipients weight, as shown by the Perugia group.6 TCD should be performed in a center with a dedicated program, where different forms of T-cell removal can be explored, including physical and immunological TCD.6,7

**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, favoring engraftment, and downregulates donor T cells, because the antibody is still in circulation at the time of transplant, and thus prevents GvHD.8–13 In vivo-TCD is indicated in programs involving alternative donor transplants, and is used in many, but not all centers performing unrelated donor grafts.8–14 In a recent randomized trial, we could prove that 15 mg/kg of rabbit antithymocyte globulin (ATG) significantly reduced the risk of grade III–IV acute GvHD from 50% in controls to 11% in ATG-treated patients (P=0.001).15 However, transplant-related mortality (TRM) and survival were unchanged due to a higher risk of lethal infections in the ATG15 mg/kg arm.15 In a retrospective analysis of 160 patients receiving or not ATG in the conditioning regimen, we could prove that ATG provided significant protection against acute and chronic GvHD, shortened time to come off immunosuppression and improved quality of life.16 Whether different schedules of administration of ATG or different agents may further improve results remains to be determined.

**Post-transplant immunosuppressive therapy**

It 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, +11.17,18 The dose CsA used in the first 10 days post-transplant may have a significant impact on leukemia control: in two prospective randomized trials, both in children and in adults, low-dose CsA (1 mg/kg) has been shown to protect patients from leukemia relapse when compared with higher doses of CsA (3 or 5 mg/kg).19,20 This was recently confirmed with a 10-year follow-up21 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 matched unrelated donor were randomized to receive CsA + MTX or FK506 + MTX.22 Acute GvHD II–IV was significantly lower (51%) in FK506-treated patients as compared to the CsA patients (70%) (P=0.0002), but this did not translate to a lower risk of chronic GvHD. The adverse events, in particular nephrotoxicity, infections or leukemia relapses were not significantly different.22 There was also no difference in survival. Therefore, both CsA + MTX and FK506 + MTX combination 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 now been introduced for GvHD prevention: its poor bioavailability when administered orally to patients who have received a conventional myeloablative conditioning may be a limiting factor. These myeloablative regimens, as well as gut decontamination, impair gastrointestinal absorption and the enterohepatic cycle. Trough levels of active metabolite in these patients have been shown to be far below concentrations measured in patients receiving MMF after solid-organ transplantation.23 The intravenous form of MMF is being used with promising results in the setting of reduced intensity transplants.24,25

**Mesenchymal stem cells**

Mesenchymal stem cell (MSC) are pluripotent stem cells capable of generating osteoblasts, myoblasts, condroblasts, tenoblasts, adipocytes and stromal cells.26 There have been several reports on the immunosuppressive effect of MSC, both in vitro27–29 and in vivo.29 The coinfusion of a large number of osteoblasts together with hematopoietic stem cells, in a mismatched mouse model, results in successful engraftment and immune reconstitution,30 whereas control animals died of GvHD or rejection. A recent trial has been completed in 40 patients with hematologic malignancies, using expanded MSC from an HLA identical sibling, and confusing these cells with a conventional unmanipulated bone marrow (BM) or peripheral blood (PB) transplant: hematopoietic reconstitution was improved and GvHD was virtually absent both in its acute and chronic form.31 The protective effect of MSC on GvHD is being tested in a prospective randomized trial. A study will also start on the use of MSC in the unrelated transplant setting.

**Inactivation of antigen-presenting cells**

Presentation of target antigens by host antigen-presenting cells (APC) has been shown to be crucial for the initiation and development of acute GvHD.32 BM chimeric mice were generated having APC unable to present class I-restricted peptides (β2M knockout, that is β2M−/−), but with class I on target tissues. These chimeric mice were then reirradiated and injected with minor HA mismatched marrow with or without 2 × 10^9 T cells. Syngeneic B6→B6 chimeras were used as controls. The β2M−/− chimeras developed no GvHD unlike the B6→B6 chimeras who developed severe GvHD.32 The age of the APC also seems
to be important, as shown by more GvHD in mice grafted with ‘older’ APC, suggesting a pathogenetic explanation for the increased risk of GvHD older recipients.33 Finally 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: this suggests that tissue destruction can be achieved with a bystander effect.34

These results suggest that depleting host APC before the conditioning regimen should abrogate GvHD. In keeping with this observation patients receiving extracorporeal photopheresis (ECP) before the conditioning regimen have a low incidence of GvHD,35 because ECP may downregulate host APC.36 Recently, the elimination of host APC in the mouse, using donor incompatible NK cells, could be followed by the infusion of mismatched T cells, without GvHD.37 It may be that cell manipulation of the graft and removal of selected cell subpopulations in the host will lead to better prophylaxis of GvHD.

Reduced intensity conditioning regimens

Reduced intensity conditioning (RIC) regimens were introduced in the late 1970s when J Hobbs used busulfan 8 mg/kg, in children with inborn errors.38 Aplastic anemia (AA) patients have been conditioned for 30 years with cyclophosphamide (CY) 200 mg/kg,39 which is nonmyeloablative and is actually used today to mobilize patients with myeloma or lymphoma (7 g/m²). In these patients, receiving a small dose of busulfan or CY alone, GvHD is less severe as compared to CY + total body irradiation (TBI), because of the persistence of host cells (mixed chimeras), which counteract GvHD.40,41 There may also be a lower level of inflammatory cytokines. RIC regimens based on low-dose TBI (2 Gy),42 low-dose busulfan,43 low-dose thiotepa44 low-dose melphalan45 or CY and fludarabine44 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. Still acute GvHD 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.45 Therefore, also in the setting of RIC regimens further improvement in preventing GvHD would be desirable. Some regimens include anti-T-cell antibodies such as alentuzumab46 or ATG.47 A randomized trial has been completed by the International Bone Marrow Transplant Registry (IBMTR) to test whether the addition of ATG to CY alone is beneficial in patients with aplastic anemia undergoing an allogeneic BM transplant from an HLA identical sibling, and the results will be presented soon.

Anti-IL2 anti-TNF antibodies

Other monoclonal antibodies (mAbs) interacting with IL2 or TNF have been tested in the clinical setting. Anti-

CD25 mAb seemed to delay the occurrence of GvHD; in a randomized trial, the administration of a CD25 mAb (in addition to CSA + MTX) appeared to decrease leukemia-free survival, in comparison to conventional GvHD prophylaxis.48 A humanized CD25 mAb, assessed in a double-blind, placebo-controlled randomized study, involving a total of 210 patients, failed to prevent GvHD or improve the outcome of unrelated HSCT recipients.49 A monoclonal antibody neutralizing TNF α has also been tested in 21 patients as GvHD prophylaxis: in a prospective trial, Hoeller and co-workers showed that GvHD could be delayed (by 10 days), although the overall grading was similar to controls.49

GvHD Treatment

First-line treatment

Corticosteroids Treatment of moderate to severe aGvHD is still unsatisfactory and is faced with a high mortality. Corticosteroids are used as first-line therapy: in a study on 453 patients who received prednisone 60 mg/m² for 14 days as first-line therapy, followed by an 8-week taper, an overall improvement was observed in 55% of the patients with durable (≥28 days) complete responses in 35%.50 The probability of survival at 1 year after initiation of therapy was 53%: favorable predictors of survival were younger age of patients, HLA identical sibling donors and GvHD prophylaxis other than ex vivo T cell depletion.51 The authors of this important study conclude 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. Whether a higher dose of corticosteroids would be more effective as first-line therapy has been assessed in a prospective trial of the Italian Cooperative Transplant Group (GITMO): 95 recipients of an HLA-identical BMT were randomized to receive intravenous 6-methylprednisolone (6Mpred) 2 mg/kg/day for 5 days or 10 mg/kg/day for 5 days as initial treatment of GvHD.52 The study showed that high-dose 6Mpred did not improve the response rate and survival, and transplant mortality was also similar in the two groups: at 1 year TRM was 32% for the 10 mg/kg group and 30% for the 2 mg/kg group. Two additional observations were made in that study: (a) despite the very early day of randomization (median day +12 from transplant), high-dose prednisolone did not prevent progression towards grade III–IV GvHD and (b) patients receiving 5 days of 6Mpred 2 mg/kg and responding (16%) had a significantly lower TRM as compared to nonresponders (46%).53 A recent survey of the IBMTR confirms that steroid refractory acute GvHD applies to a patient not responding to prednisone 2 mg/kg given on five consecutive days.54 Primary treatment of acute GvHD should be prednisone or 6Mpred 2 mg/kg/day for 5 days: patients responding should taper steroid therapy. Patients not responding are eligible for second-line treatment.
Second-line treatment

Conventional dose antithymocyte globlin  It is one treatment option for steroid refractory patients. In a prospective study 42 MUD-BMT recipients were given steroids as first-line therapy, followed by antithymocyte globlin ATG after prednisone failure in 22 of them.52 Prednisone treatment led to improvement in 10 out of 41 (24%) of patients, while secondary treatment with ATG caused improvement in four out of 21 (19%). This high failure rate suggests the need of early ATG treatment of acute GvHD.52 It was suggested that an extended previous corticosteroid treatment may select a clone of resistant T cells that could be insensitive to a later corticosteroid treatment when grade II–IV aGVHD developed53 This hypothesis appeared to be confirmed by the finding that glucocorticoids prophylaxis is associated with a higher risk of GvHD and higher treatment failure. Because of these unsatisfactory data, Cragg et al.54 performed a randomized trial comparing the combination ATG/prednisone versus prednisona as initial treatment of GvHD. They failed to show improved control of GvHD and obtained no significant difference in survival. In the ATG/prednisone arm, there was an increased incidence of infections. The authors used an aggressive immunosuppressive therapy in the ATG/prednisone arm with high-dose ATG (15 mg/kg ATG bid) plus 20 mg/mq prednisone bid on each of five consecutive days.54 Therefore, high-dose ATG seems ineffective for the management of established acute GvHD, especially if treatment is started beyond 2 weeks from initial symptoms of GvHD: if ATG is given within 14 days results may be more promising.55

Low-dose ATG

Recent experimental data suggest that rabbit ATG in low doses (10 µg/ml) may induce incomplete T-cell activation, apoptosis and hyporesponsiveness.56–57 We have therefore designed a pilot study for treatment of acute GvHD grade II or greater, with low-dose alternate day rabbit ATG and methylprednisolone. In all, 15 patients received ATG as first-line treatment, within 14 days from diagnosis of GvHD, and 12 patients received ATG as second-line therapy, beyond day 14; responses were significantly superior in patients treated early and TRM reduced (P = 0.02); the actuarial 3 year survival was, respectively, 45% versus 23% (P = 0.06). In keeping with these data, a recent study confirms that response and survival are superior for patients given ATG within 14 days.55 A prospective trial within the Italian transplant group (GITMO) has been opened to test whether low-dose ATG with steroids is superior to steroids alone for the treatment of GvHD.

Anti-CD147 monoclonal antibody

Deeg and coworkers have most recently 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%.58 A randomized trial is being set up to test anti-CD147 for the treatment of acute GvHD.

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 BM transplant (BMT), based on serum bilirubin and blood urea nitrogen levels.59 We have recently revised the scoring system with the inclusion of day +7 cholinesterase, gammaglutamyltransferase, total protein together with cell dose and donor type.60 One possible approach to reduce the risk of GvHD and TRM, is pre-emptive treatment with T-cell antibodies. 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. This is in keeping with a previous randomized 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.61 We are now testing this approach in a prospective randomized trial in patients undergoing an alternative donor transplant.

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 human and animal models with some positive results, although anecdotal.62 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 action. During induction phase the administration of IL12 inhibits GvHD.63 In the same mouse model if IL12 is given later after transplant, there is enhancement of GvHD by induction of host-derived IFNγ.64 T cells from IFNγ knockout mice surprisingly cause a more virulent GvHD,65 suggesting a protective effect of IFNγ. In a different mouse P→F1 model the lack of IFNγ T cells delays GvHD.66 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 what T cells need to be killed? In a recent paper Liu and coworkers have tried to answer these questions, by using a transgene mouse model. In these animals the HSV-tk transgene is controlled either by the IL2 or by the IL4 promoters (IL2-tk and IL4-tk) thus allowing investigations in the role of Th1 and Th2.67 Although both thyro sine kinases are expressed very early upon T-cell activation, when T cells are polarized towards Th1, only IL2-tk transgene is expressed, and the opposite is true under Th2 polarization.68,69 Liu and coworkers have been able to show, convincingly, that clinical GvHD (weight loss and death) as well as histologic lesions regressed in gancyclovir-treated IL2-tk and IL4-tk recipients, consistent with GCV-induced death of dividing alloreactive donor T cell.69 This supports a role for both Th1 and Th2 in the generation of GvHD. Conditional ablation of Th1 or Th2 polarized T cells could pave the road for effective treatment of GvHD (ablation of Th2) still leaving intact GvL (Th1).

**Acute GvHD and reduced intensity regimens**

There are several reasons why acute GvHD may be increasing with the current allogeneic transplant practice: older donor/patients age, greater use of unrelated or family mismatched donors, greater use of peripheral blood cells, early discontinuation of in vivo immunosuppression and early use of donor lymphocyte infusions (DLI). The reduced intensity regimens (RIC) seem to have all of these risk factors for acute GvHD, since they are designed to maximize the immune effect of the graft in an attempt to control the underlying malignant disease. Several questions need to be answered in this context: timing and intensity of in vivo immunosuppression, timing of cyclosporin discontinuation, timing of DLI and use of T-cell antibodies. It is interesting that Russell and coworkers have taken a different approach: they decided to use ablative doses of intravenous busulfan combined with fludarabine and have tried to maximize GvHD prevention with rabbit ATG in the conditioning regimen and CsA MTX for postgraft immunosuppression.70 The program they call FLU-BUP is given up to the age of 65 years, for patients with hematologic malignancies, and yields a TRM of 4% in HLA identical siblings and 20% in unrelated transplants.

**Viral infections: CMV and EBV**

Viral infections are frequent in patients with GvHD. Cytomegalovirus (CMV) has been the object of numer-
References


