Graft-versus-host disease

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Haemopoietic-cell transplantation (HCT) is an intensive therapy used to treat high-risk haematological malignant disorders and other life-threatening haematological and genetic diseases. The main complication of HCT is graft-versus-host disease (GVHD), an immunological disorder that affects many organ systems, including the gastrointestinal tract, liver, skin, and lungs. The number of patients with this complication continues to grow, and many return home from transplant centres after HCT requiring continued treatment with immunosuppressive drugs that increases their risks for serious infections and other complications. In this Seminar, we review our understanding of the risk factors and causes of GVHD, the cellular and cytokine networks implicated in its pathophysiology, and current strategies to prevent and treat the disease. We also summarise supportive-care measures that are essential for management of this medically fragile population.

Introduction

The number of allogeneic haemopoietic-cell transplantations (HCTs) continues to rise, with more than 25 000 procedures undertaken annually. The graft-versus-leukaemia or graft-versus-tumour effect during this procedure effectively eradicates many haematological malignant diseases. Development of novel strategies that use donor leucocyte infusions, non-myeloablative conditioning, and umbilical-cord blood transplantation has helped expand the indications for allogeneic HCT over the past few years, especially for older patients. Improvements in infectious prophylaxis, immunosuppressive treatments, supportive care, and DNA-based tissue typing have also contributed to enhanced outcomes after the technique. Yet, the major complication of allogeneic HCT—graft-versus-host disease (GVHD)—remains lethal and limits use of this important procedure. In view of current trends, the number of transplants from unrelated donors is expected to double within the next 5 years, substantially increasing the population of patients with GVHD. In this Seminar, we review advances made in identification of genetic risk factors and pathophysiology of this major HCT complication and its prevention, diagnosis, and treatment.

Cause and clinical features

50 years ago, Billingham formulated three requirements for development of GVHD: (1) the graft must contain immunologically competent cells; (2) the recipient must express tissue antigens that are not present in the transplant donor; and (3) the patient must be incapable of mounting an effective response to eliminate the transplanted cells. We know now that the immunologically competent cells are T cells and that GVHD can develop in various clinical settings when tissues containing T cells (blood products, bone marrow, and solid organs) are transferred from one person to another who is not able to eliminate those cells. Patients whose immune systems are suppressed and who receive white blood cells from another individual are at especially high risk for the disease.

GVHD arises when donor T cells respond to genetically defined proteins on host cells. The most important proteins are human leucocyte antigens (HLAs), which are highly polymorphic and are encoded by the major histocompatibility complex (MHC). Class I HLA (A, B, and C) proteins are expressed on almost all nucleated cells of the body at various densities. Class II proteins (DR, DQ, and DP) are mainly expressed on haemopoietic cells (B cells, dendritic cells, and monocytes), but their expression can be induced on umbilical-cord blood grafts (see Clinical features of acute GVHD). High-resolution DNA typing of HLA genes with PCR-based techniques has now largely replaced earlier methods. The frequency of acute GVHD is directly related to the degree of mismatch between HLA proteins, and thus ideally, donors and recipients are matched at HLA A, B, C, and DRB1 (referred to as 8/8 matches), but mismatches can be tolerated for umbilical-cord blood grafts (see Clinical features of acute GVHD).

Despite HLA identity between a patient and donor, about 40% of recipients of HLA-identical grafts develop systemic acute GVHD that needs treatment with high-dose steroids. This disorder is due to genetic differences that lie outside the HLA loci and that encode proteins referred to as minor histocompatibility antigens. Some minor histocompatibility antigens, such as HY and HA-3, are expressed on all tissues and are targets for both GVHD and graft-versus-leukaemia. Others, such as HA-1 and HA-2, are expressed most abundantly on haemopoietic cells (including leukaemic cells) and could, therefore, induce an enhanced graft-versus-leukaemia effect with diminished GVHD.

Search strategy and selection criteria

We searched PubMed and Medline with the term “GVHD” and cross-referenced it with the following words: “clinical”, “cytokines”, “MHC”, “HLA antigens”, “biology”, and “immunology”. We included mostly peer-reviewed original and review journal articles published within the past decade, except for seminal articles that initially described the clinical features. All non-peer-reviewed manuscripts, supplements, and textbooks were excluded.
Polymorphisms in both donors and recipients of cytokines that have a role in the classic cytokine storm of GVHD (see Pathophysiology of acute GVHD) have been implicated as risk factors for the disorder. Tumour necrosis factor (TNF) α, interleukin 10, and interferon γ variants have correlated with GVHD in some, but not all, studies. Genetic polymorphisms of proteins connected with innate immunity, such as nucleotide oligomerisation domain 2 and keratin 18 receptors, have also been associated with the disorder. Future strategies to identify the best possible transplant donor will probably incorporate both HLA and non-HLA genetic factors.

Clinical features of acute GVHD

On the basis of early work, acute GVHD was defined as arising before day 100 post-transplant, whereas chronic disease happened after that time. This definition is far from satisfactory, and a National Institutes of Health classification includes late-onset acute GVHD (after day 100) and an overlap syndrome with features of both acute and chronic disorder. Late-onset acute GVHD and the overlap syndrome arise with greater frequency after reduced-intensity conditioning, an increasingly widespread technique (see Prevention of GVHD). Panel 1 shows the clinical manifestations of acute GVHD. In a comprehensive review, Martin and colleagues noted that at onset of acute GVHD, affected regions included skin (81% of patients), gastrointestinal tract (54%), and liver (50%).

Skin is most frequently affected and is usually the first organ involved, generally coinciding with engraftment of donor cells. The characteristic maculopapular rash is pruritic and can spread throughout the body, sparing the scalp (figure 1). In severe cases the skin can blister and ulcerate. Apoptosis at the base of epidermal rete pegs is a characteristic pathological finding. Other features include dyskeratosis, exocytosis of lymphocytes, satellite lymphocytes adjacent to dyskeratotic epidermal keratinocytes, and perivascular lymphocytic infiltration in the dermis.

Gastrointestinal-tract involvement of acute GVHD usually presents as diarrhoea but can also include vomiting, anorexia, abdominal pain, or a combination when severe. Diarrhoea in GVHD is secretory and usually voluminous (>2 L per day). Bleeding, which has poor prognosis, happens as a result of mucosal ulceration, but patchy involvement of mucosa generally leads to a normal appearance on endoscopy. Radiological findings of the gastrointestinal tract include luminal dilatation with thickening of the wall of the small bowel (ribbon sign on CT) and air or fluid levels suggestive of an ileus. Histological features include patchy ulcerations, apoptotic bodies in the base of crypts, crypt abscesses, and loss and flattening of surface epithelium.

Liver disease caused by GVHD can be difficult to distinguish from other causes of liver dysfunction after bone-marrow transplantation, such as veno-occlusive disease, toxic drug effects, viral infection, sepsis, or iron overload. The histological features of hepatic GVHD are endothelialitis, lymphocytic infiltration of the portal areas, pericholangitis, and bile-duct destruction. However, biopsy specimens of liver are taken rarely because thrombocytopenia early after transplantation greatly increases the risks of the biopsy procedure, making the diagnosis of GVHD one of exclusion.

Severity of acute GVHD is ascertained by the extent of involvement of the three main target organs. Overall grades are I (mild), II (moderate), III (severe), and IV (very severe). Severe GVHD has poor prognosis, with 25% long-term survival (5 years) for grade III disease and 5% for grade IV.
Prevalence of acute GVHD is directly related to the degree of mismatch between HLA proteins. It ranges from 35–45% in recipients of full-matched sibling donor grafts to 60–80% in people receiving one-antigen HLA-mismatched unrelated-donor grafts. The same amount of mismatch causes diminished GVHD with umbilical-cord blood grafts, and frequency of acute GVHD is low after transplantation of partly matched umbilical-cord blood units (35–65%).

Pathophysiology of acute GVHD

Two important principles should be considered with respect to the pathophysiology of acute GVHD. First, the disease is indicative of exaggerated but typical inflammatory mechanisms mediated by donor lymphocytes infused into the recipient, in whom they function appropriately in view of the foreign environment they encounter. Second, the recipient’s tissues that stimulate donor lymphocytes have usually been damaged by underlying disease, previous infections, and the transplant conditioning regimen. As a result, these tissues produce molecules such as proinflammatory cytokines and chemokines, which increase expression of key receptors on antigen-presenting cells (APCs), thereby enhancing cross-presentation of polypeptide proteins (eg, minor histocompatibility antigens) to the donor immune cells that mediate GVHD.

Mouse models have been central to identification and understanding of pathophysiological mechanisms of GVHD, and work undertaken in dogs has been vital for development of clinically useful strategies for GVHD prophylaxis and treatment advances in donor leucocyte infusions. Largely on the basis of these experimental data, progression of acute GVHD can be summarised in three sequential steps or phases: (1) activation of APCs; (2) donor T-cell activation, proliferation, differentiation, and migration; and (3) target tissue destruction.

The first step entails activation of APCs by the underlying disease and the HCT conditioning regimen. Damaged host tissues respond by producing so-called danger signals, including proinflammatory cytokines (eg, TNFα and interleukins 1 and 6), chemokines, and amplified expression of adhesion molecules, MHC antigens, and costimulatory molecules on host APCs. Findings of a report showed that 1 week after HCT, increased amounts of TNFα receptor 1—a surrogate marker for TNFα—correlated strongly with later development of GVHD. Injury to the gastrointestinal tract from conditioning is especially important because it allows for systemic translocation of additional inflammatory stimuli, such as microbial products including lipopolysaccharide or other pathogen-associated molecular patterns, that further enhance activation of host APCs.

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The secondary lymphoid tissue in the gastrointestinal tract is probably the initial site of interaction between activated APCs and donor T cells. These observations have led to an important clinical strategy to diminish acute GVHD by reducing the intensity of the conditioning regimen. Experimental GVHD can also be decreased...
by manipulation of distinct subsets of APCs.\textsuperscript{55,56}
Furthermore, non-haemopoietic stem cells, such as mesenchymal stromal cells, can reduce allogeneic T-cell responses and ameliorate GVHD, although the mechanism for such inhibition remains unclear.\textsuperscript{57}

The idea that amplified activation of host APCs increases the risk for acute GVHD unifies several seemingly disparate clinical associations with that risk, such as advanced stages of malignant disease, more intense transplant conditioning regimens, and history of viral infection. APCs detect infections with receptors on their cell surfaces, such as Toll-like receptors, which recognize conserved molecular patterns of microbes.\textsuperscript{27,58} Toll-like receptors specific for viral DNA or RNA activate APCs and could boost GVHD, providing a potential mechanistic basis for enhanced disease associated with viral infections such as cytomegalovirus.\textsuperscript{59}

The core of the graft-versus-host reaction is the second step, in which donor T cells proliferate and differentiate in response to host APCs (figure 3). The danger signals generated in the first phase augment this activation, at least in part, by increasing expression of costimulatory molecules.\textsuperscript{60} Blockade of costimulatory pathways to prevent GVHD is successful in animal models, but this approach has not yet been tested in large clinical trials.\textsuperscript{2}

In mouse models, in which genetic differences between donor and recipient strains can be tightly controlled, CD4+ cells induce acute GVHD to MHC class II differences and CD8+ cells induce acute disease to class I differences.\textsuperscript{61,62} In most HLA-identical HCTs, both CD4+ and CD8+ subsets respond to minor histocompatibility antigens and can cause GVHD in HLA-identical procedures.

Regulatory T cells can suppress proliferation of conventional T cells and prevent GVHD in animal models when added to donor grafts containing conventional T cells,\textsuperscript{63} but use of regulatory T cells in clinical acute GVHD will need enhanced techniques to identify and expand them. Natural killer T-cell 1.1+ subsets from the host and donors have also been shown to modulate acute GVHD.\textsuperscript{64} In a clinical trial of total lymphoid irradiation (as conditioning), GVHD was reduced significantly and host natural killer T-cell function was amplified.\textsuperscript{65}

Activation of immune cells results in rapid intracellular biochemical cascades that induce transcription of genes for many proteins, including cytokines and their receptors. T-helper 1 cytokines (interferon γ, interleukin 2, and TNFα) are released in large amounts during acute GVHD. Production of interleukin 2 by donor T cells remains the main target of many current clinical therapeutic and prophylactic approaches to GVHD, such as cyclosporine, tacrolimus, and monoclonal antibodies directed against this cytokine and its receptor.\textsuperscript{66} However, emerging data indicate an important role for interleukin 2 in the generation and maintenance of CD4+CD25+ regulatory T cells, suggesting that prolonged interference with this cytokine could unintentionally stop development of long-term tolerance after allogeneic HCT.\textsuperscript{66}

Interferon γ has many functions and can either amplify or reduce GVHD.\textsuperscript{67,68} It could boost disease by increasing expression of molecules such as chemokine receptors, MHC proteins, and adhesion molecules; it also raises the sensitivity of monocytes and macrophages to stimuli such as lipopolysaccharide and accelerates intracellular cascades in response to these stimuli.\textsuperscript{69} Early polarisation of donor T cells so that they secrete less interferon γ and more interleukin 4 can also attenuate experimental acute GVHD.\textsuperscript{70} Interferon γ might amplify GVHD by direct damage to epithelium in the gastrointestinal tract and skin and by induction of immunosuppression by generation of nitric oxide.\textsuperscript{71} By contrast, this cytokine could suppress GVHD by hastening apoptosis of activated donor T cells.\textsuperscript{67,72} This complexity means manipulation of interferon γ could have diverse effects in vivo, making the cytokine a challenging target with respect to therapeutic intervention.

Interleukin 10 has a key role in suppression of immune responses, and clinical data suggest it might regulate acute GVHD.\textsuperscript{73} Transforming growth factor β, another suppressive cytokine, can subdue acute GVHD but exacerbate chronic disease.\textsuperscript{73} Thus, timing and duration of secretion of any given cytokine could establish the specific effects of that molecule on GVHD severity.
The third effector phase of the graft-versus-host process (figure 3) is a complex cascade of cellular mediators (such as cytotoxic T lymphocytes and natural killer cells) and soluble inflammatory agents (eg, TNFα, interferon γ, interleukin 1, and nitric oxide).2,29 These molecules work synergetically to amplify local tissue injury and further promote inflammation and target tissue destruction.

The cellular effectors of acute GVHD are mainly cytotoxic T lymphocytes and natural killer cells.49 Cytotoxic T lymphocytes that prefer to use the Fas and FasL pathway of target lysis seem to predominate in GVHD liver damage (hepatocytes express large amounts of Fas) whereas cells that use the perforin and granzyme pathways are more important in the gastrointestinal tract and skin.2,74 Chemokines direct migration of donor T cells from lymphoid tissues to the target organs in which they cause damage. Macrophage inflammatory protein 1α and other chemokines (such as CCL2–CCL5, CXCL2, CXCL9, CXCL10, CXCL11, CCL17, and CCL27) are overexpressed and enhance homing of cellular effectors to target organs during experimental GVHD.7 Expression of integrins, such as α4β7 and its ligand MADCAM1, is also important for homing of donor T cells to Peyer’s patches during intestinal GVHD.12,76,77

Microbial products such as lipopolysaccharide, which leak through damaged intestinal mucosa or skin, can stimulate secretion of inflammatory cytokines through Toll-like receptors.49,78 The gastrointestinal tract is especially susceptible to damage from TNFα, and the gastrointestinal tract has a major role in amplification and propagation of the cytokine storm characteristic of acute GVHD.49 TNFα can be produced by both donor and host cells and it acts in three different ways: (1) it activates APCs and enhances alloantigen presentation; (2) it recruits effector cells to target organs via induction of inflammatory chemokines; and (3) it directly causes tissue necrosis (as its name suggests).79–81

Prevention of GVHD
On the basis of evidence from animal models for the central role of T cells in initiation of GVHD, many clinical studies of T-cell depletion as prophylaxis for the disease were undertaken in the 1980s and 1990s. Three main depletion strategies were studied: (1) negative selection

Figure 3: Pathophysiology of acute GVHD
IL 1=interleukin 1, IFN γ=interferon γ, LPS=lipopolysaccharide. Treg=regulatory T cell. Th1=T-helper 1 cell. CTL=cytotoxic T lymphocyte.
of T cells ex vivo; (2) positive selection of CD34+ stem cells ex vivo; and (3) antibodies against T cells in vivo.46
Most approaches showed substantial limitation of both acute and chronic GVHD.45–48 Unfortunately, the lowest frequency of severe GVHD was offset by high rates of graft failure, relapse of malignant disease, infections, and Epstein-Barr virus-associated lymphoproliferative disorders. Negative-selection purging strategies with various antibodies against T cells achieved similar long-term results irrespective of the breadth of antibody specificity.46–48 Findings of one large registry study showed that purging techniques that used antibodies with broad specificities produced inferior leukaemia-free survival than standard immunosuppression in patients receiving unrelated donor transplants.89

Several research groups have investigated partial T-cell depletion, either by elimination of specific T-cell subsets (eg, CD8+) or by titration of the dose of T cells present in the inoculum.90–92 None of these approaches, however, has been shown convincingly to be the best strategy that enhances long-term survival.

Alemtuzumab is a monoclonal antibody that binds CD52, a protein expressed on a broad range of leukocytes including lymphocytes, monocytes, and dendritic cells. Its use in a phase II trial of GVHD prophylaxis lowered incidence of acute and chronic GVHD after reduced-intensity transplant.93 In two prospective studies, patients who received alemtuzumab rather than methotrexate showed significantly lower rates of acute and chronic GVHD,94 but they had more infectious complications and higher rates of relapse, so no overall survival benefit was recorded. Alemtuzumab might also contribute to graft failure when used with minimum-intensity conditioning regimens.95

An alternative strategy to T-cell depletion attempted to induce anergy in donor T cells by ex-vivo antibody blockade of costimulatory pathways before transplantation. Findings of a small study of this approach in patients undergoing haploidentical HCT was quite encouraging, but they have not yet been replicated.89 Thus, the focus of most preventive strategies remains pharmacological manipulation of T cells after transplant.

Administration of antibodies against T cells in vivo as GVHD prophylaxis has also been tested extensively. The best studied drugs are anti-thymocyte globulin or anti-lymphocyte globulin preparations. These serum samples, which have high titres of polyclonal antibodies, are made by immunisation of horses or rabbits to thymocytes or lymphocytes, respectively. A complicating factor in establishing the role of these polyclonal serum samples in transplantation is the observation that even different brands of the same class exert diverse biological effects.96 However, the side-effects of anti-thymocyte globulin and anti-lymphocyte globulin infusions are similar across different preparations and include fever, chills, headache, thrombocytopenia (from cross-reactivity to platelets), and, infrequently, anaphylaxis.

In retrospective studies, rabbit anti-thymocyte globulin reduced the frequency of GVHD in related-donor haemopoietic stem-cell transplant recipients without seeming to enhance survival.97,98 In patients receiving unrelated-donor haemopoietic stem cells, addition of anti-lymphocyte globulin to standard GVHD prophylaxis prevented severe GVHD effectively but did not result in better survival because of increased infections.99 In a long-term follow-up study, however, pretransplant anti-thymocyte globulin provided significant protection against extensive chronic GVHD and chronic lung dysfunction.100

The primary pharmacological strategy to prevent GVHD is inhibition of the cytoplasmic enzyme calcineurin, which is important for activation of T cells. The calcineurin inhibitors cyclosporine and tacrolimus have similar mechanisms of action, clinical effectiveness, and toxic effects, including hypomagnesaemia, hyperkalaemia, hypertension, and nephrotoxicity.102 Serious side-effects include transplant-associated thrombotic microangiopathy and neurotoxic effects that can lead to premature discontinuation. Although clinically similar to thrombotic thrombocytopenic purpura, transplant-associated thrombotic microangiopathy does not respond reliably to therapeutic plasmapheresis, carries a high mortality rate, and removal of the offending agent does not always result in improvement.103 Posterior reversible encephalopathy syndrome includes mental status changes, seizures, neurological deficits, and characteristic findings on MRI; this syndrome has been seen in 1–2% of patients undergoing HCT and taking calcineurin inhibitors.104 Side-effects of these drugs fall as the dose is tapered, usually 2–4 months after transplantation.

Calcineurin inhibitors are usually administered in combination with other immunosuppressants, such as methotrexate, which is given at low doses in the early post-transplant period.105 The toxic effects of methotrexate (neutropenia and mucositis) have led some investigators to replace it with mycophenolate mofetil. In a prospective randomised trial, patients who received mycophenolate mofetil as part of GVHD prophylaxis had significantly less severe mucositis and more rapid neutrophil engraftment than did those who received methotrexate.106 Frequency and severity of acute GVHD was similar between the two groups, but the study closed early because of superiority of the mycophenolate mofetil arm with respect to reduced mucositis and speed of haemopoietic engraftment. A desire for faster neutrophil engraftment has led to use of mycophenolate mofetil in umbilical-cord blood transplants for which graft failure is a major concern.107 This drug is also sometimes used after reduced-intensity conditioning regimens for similar reasons.96,108

Sirolimus is an immunosuppressant that is structurally similar to tacrolimus but does not inhibit calcineurin. In phase II trials, sirolimus was very effective in combination with tacrolimus;109 the drug damages endothelial cells, however, and it might enhance transplant-associated...
Reduced-intensity conditioning regimens attempt to suppress the host immune system sufficiently so that donor T cells can engraft and then ablate the lymphohaemopoietic compartment of the recipient. The term non-myeloablative is therefore somewhat misleading. Reduced-intensity conditioning regimens diminish tissue damage and lead to decreased amounts of inflammatory cytokines, which are important in the initiation of GVHD pathophysiology; this effect could account for the reduced frequency of severe GVHD after reduced-intensity conditioning versus full-intensity conditioning used in historical controls.53,54,93,111 Onset of acute GVHD can be delayed after reduced-intensity conditioning until after day 100, however, and acute disease could present simultaneously with elements of chronic GVHD (known as overlap syndrome).105–113

Treatment of acute GVHD
GVHD first develops, generally, in the second month after HCT during calcineurin-based prophylaxis.114 Steroids, with their potent anti-lymphocyte and anti-inflammatory activity, are the gold standard for treatment of GVHD. Many centres treat mild GVHD of the skin (grade I) with topical steroids alone, but for more severe disease and any degree of visceral GVHD involvement high-dose systemic steroids are usually initiated. Administration of steroids results in complete remission in less than half of patients,115 and more severe GVHD is less likely to respond to treatment.116 In a prospective randomised study, addition of anti-thymocyte globulin to steroids as primary treatment did not increase the response rate.116 In a retrospective study, use of anti-thymocyte globulin in patients who showed early signs of steroid resistance was beneficial,117 but not all study findings show such benefit, and this antibody preparation is not used as standard because of raised infection risks.101,127 Infusion of mesenchymal stromal cells—expanded in culture either from the original HCT donor or from a third party—is a promising approach, which produced 55% complete responses in a phase II study of patients with steroid-resistant GVHD.118

An increasingly frequent treatment for GVHD is extracorporeal photopheresis. During this procedure, the patient’s white blood cells are gathered by apheresis, incubated with the DNA-intercalating agent 8-methoxypsoralen, exposed to ultraviolet light, and returned to the patient. Extracorporeal photopheresis is known to induce cellular apoptosis, which has strong anti-inflammatory effects in several systems, including prevention of rejection of solid organ grafts.119 Work done in animals shows that extracorporeal photopheresis reverses acute GVHD by increasing the number of regulatory T cells.120 Data from a phase II clinical study of steroid-dependent or steroid-refractory GVHD showed resolution of disease in most patients, with 50% long-term survival in this very-high-risk group.120 Randomised multicentre studies of this approach are needed to establish its place in management of acute GVHD.

Another strategy to treat GVHD is blockade of the inflammatory cytokine TNFα. TNFα can activate APCs, recruit effector cells, and cause direct tissue damage (see Pathophysiology of acute GVHD).121 Data from a phase II trial of etanercept (solubilised TNFα receptor 2) showed significant effectiveness of the drug when added to systemic steroids as primary treatment for acute GVHD. 70% of patients had complete resolution of all GVHD symptoms within 1 month, with 80% complete responses in the gastrointestinal tract and skin. The researchers also reported that concentrations in plasma of TNFα receptor 1 were a significant biomarker for clinical GVHD.122

Treatment of chronic GVHD
By contrast with acute GVHD, the pathophysiology of chronic GVHD remains poorly understood and the disease is treated with various immunosuppressive agents. The response of chronic GVHD to treatment is unpredictable, and mixed responses in different organs can take place in the same patient. Confounding variables such as infection and comorbidities also make responses hard to measure. Use of corticosteroids (with or without a calcineurin inhibitor) is the standard of care, but findings of a randomised trial of more than 300 patients with chronic GVHD noted no difference between cyclosporine plus prednisone versus prednisone alone.123 Chronic immunosuppressants, especially those containing steroids, are highly toxic and result in deaths from infection. Many second-line treatments have been studied, but none has achieved widespread acceptance. As mentioned in the Treatment of acute GVHD section, extracorporeal photopheresis shows some promise, with relevant response rates in high-risk patients. The best responses were seen in skin, liver, oral mucosa, eye, and lung.124 This observation is especially pertinent because lung GVHD has the potential to be a particularly devastating complication of chronic GVHD. Inhaled high-dose steroids, when added to existing immunosuppressant regimens, have stabilised the pulmonary function of patients with bronchiolitis obliterans in a small trial.125 If other treatments fail, lung transplantation might be the only remaining therapeutic option.126

Essential supportive care in GVHD patients
Meticulous supportive care is vital for patients with both acute and chronic GVHD owing to the extended duration of immunosuppressive regimens and because the many drugs administered could have synergistic toxic effects. Such care includes extensive infectious prophylaxis, early interventions in cases of suspected infections, and prophylaxis against non-infectious side-effects of drugs (table). These complications usually need rapid responses.
Viral infections are frequent in people with GVHD. Cytomegalovirus causes interstitial pneumonia and gastritis. Patients who are at risk should have their blood monitored several times a month. Techniques that directly detect virus should be undertaken, such as cytomegalovirus PCR or pp65 antigen, and evidence of increased viral load should prompt preemptive treatment with ganciclovir or foscarnet before clinical manifestations of disease. Shingles is not uncommon and aciclovir prophylaxis could be beneficial. Patients and caregivers should receive vaccinations against influenza, and treatment with neuraminidase inhibitors (influenza), other antivirals

<table>
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<tr>
<th>Clinical symptoms</th>
<th>Routine monitoring</th>
<th>Prophylaxis</th>
<th>Recommended intervention</th>
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<td><strong>Bacterial infections</strong></td>
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<tr>
<td>Catheter-related</td>
<td>Fever, chills, pain, erythema</td>
<td>Assessment of catheter sites</td>
<td>Sterile dressing, regular line maintenance</td>
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<tr>
<td>Other</td>
<td>Fever, chills, sepsis symptoms</td>
<td>Clinical signs, chest radiograph or CT scan for possible pneumonia</td>
<td>Antibiotics in high-risk patients (high-dose corticosteroids or asplenia), intravenous immunoglobulin if IgG level &lt;400 g/L</td>
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| **Viral infections** | | | |
| Cytoemgalovirus | Gastroenteritis, interstitial pneumonia | Blood cytomegalovirus PCR or pp65 antigen levels | Pre-emptive treatment in patients with reactivation | Antiviral treatment (ganciclovir, valganciclovir, or foscarnet) |
| Respiratory viruses | Symptoms of upper or lower respiratory-tract infections | Clinical monitoring | Annual influenza vaccination (starting 6 months post HCT), vaccination of caregivers | Early treatment with neuraminidase inhibitors (influenza), other antivirals |
| Varicella-zoster virus | Vesicular skin lesions | Clinical monitoring | Aciclovir prophylaxis | Treatment doses of antivirals |

| **Fungal and other infections** | | | |
| Aspergillosis, other emerging fungal infections | Pulmonary lesions, sinuses, skin nodules | Galactomannan, assays in high-risk patients, CT scan if signs of infection | Voriconazole, or posaconazole prophylaxis in high-risk patients (eg, high-dose steroids) | Antifungal treatment |
| Candida | Thrush, pulmonary lesions | Clinical examination, CT scan if signs of infection | Fluconazole (aspergillus prophylaxis protects against candida too) | Antifungal treatment |
| Pneumocystis | Fever, hypoxia, respiratory distress | Clinical assessment | Cotrimoxazole, or pentamadine until 1 month off immunosuppression | Treatment doses of anti-PCP drugs |

| **Other toxic effects of immunosuppressive agents** | | | |
| Calcineurin inhibitors | Tremor | Clinical assessment, drug concentrations | Adjust dose to desired trough levels | |
| Calcineurin inhibitors | Neurotoxic effects | Assess mental status | Stop calcineurin inhibitors | |
| Calcineurin inhibitors | Renal impairment | Creatinine levels and glomerular filtration rate | Adequate fluid uptake (about 3 L per day) | Intraavenous fluids |
| Calcineurin inhibitors | Hypertension | Blood pressure monitoring | Stop calcineurin inhibitors, plasmapheresis | |
| Calcineurin inhibitors | Transplant-associated microangiopathy | Assess blood smear for haemolysis, schistocytes | Stop calcineurin inhibitors, plasmapheresis | |
| Corticosteroids | Cushing’s disease symptoms | Clinical assessment | Taper as recommended | |
| Corticosteroids | Diabetes | Blood glucose levels | Nutritional guidance | Insulin treatment |
| Corticosteroids | Osteoporosis | Assessment of bone density | Calcium or vitamin D supplementation | Bisphosphonate treatment for osteoporosis |
| Corticosteroids | Myopathy | - | Physiotherapy | |

**Late graft failure**

| Blood disorders | Bleeding symptoms, anaemia | Blood counts | Stop or switch drugs | Growth factors (granulocyte colony-stimulating factor), erythropoetin, transfusions |

Table: Recommendations for supportive care

**PCP=Pneumocystis jirovecii**

to prevent serious or irreversible damage and are best handled by close collaboration between the primary doctor and the transplant specialist.

All patients should receive at least fluconazole as prophylaxis against fungal infections. Invasive moulds, especially aspergillus, are typical with prolonged steroid use.29 Prophylaxis with voriconazole or posaconazole should be considered for these individuals. Usual sites of infection are the lungs, sinuses, brain, and skin,30 and serial galactomannan assays could aid in early detection.31 Candida can cause lesions in the lung, liver, and spleen, which might need screening with ultrasonography. Pneumocystis is another opportunistic infection that should receive cotrimoxazole (bactrim) prophylaxis.30
Patients with GVHD sometimes have IgG2 and IgG4 subclass deficiencies despite usual amounts of IgG, making them susceptible to infections with encapsulated organisms. Treatment of severe hypogammaglobulinemia with intravenous immunoglobulin is standard in many centres,134 but the level that triggers replacement varies considerably between transplant specialists. Supporting evidence for routine use of intravenous immunoglobulin as prophylaxis is scarce.135 but patients should receive routine prophylaxis (penicillin or its equivalent) because of increased risk for streptococcal sepsis.136 Pneumococcal conjugate and Haemophilus influenzae vaccine might provide additional protection and are recommended for all patients, including those with chronic GVHD.130,137

The sites of any indwelling catheters should be assessed regularly and early treatment of a suspected infection initiated. Early signs or symptoms of septic shock, such as shaking chills or low blood pressure, need prompt assessment with chest radiography, CT scan, or both, blood culture, and treatment with broad-spectrum antibiotics because shock can progress rapidly in these patients.

Chronic immunosuppressant treatment has many toxic effects. Diabetes (which further increases risks for infection), muscle weakness, osteoporosis, avascular necrosis (usually requiring joint replacement), and other cushingoid features are typical with chronic steroid use. Frequent monitoring of blood glucose and screening of bone density are recommended, and treatment includes insulin, calcium, vitamin D, and bisphosphonates.138,139

Calcineurin inhibitors frequently cause renal impairment, hypertension, and neurological parapathies. Standard supportive care includes blood pressure monitoring, assessment of renal function, and monitoring of drug concentrations in blood, which should be maintained within therapeutic ranges. To prevent renal dysfunction, most centres recommend vigorous oral outpatient hydration. Some patients are unable to tolerate calcineurin inhibitors and need different immuno-suppressive drugs altogether.

Cytopenias sometimes require treatment with growth factors such as granulocyte-colony-stimulating factor or cessation of the offending agent. These approaches should always be taken in close consultation with a transplant specialist.

Future directions
As allogeneic transplantation becomes an increasingly attractive therapeutic option, need for novel approaches to GVHD has accelerated. The number of patients receiving transplants from unrelated donors is expected to double in the next 5 years, substantially boosting the population of patients with GVHD.

The advent of reduced-intensity conditioning regimens has diminished transplant-related mortality and lengthened the period during which acute GVHD can develop (many new cases present up to day 200), and need for close monitoring of individuals in this period has risen. Patients have typically returned to the care of their primary haematologists by this time, increasing the need for these doctors to collaborate with transplant specialists in the management of GVHD and its complications.

Identification of biomarkers for GVHD with diagnostic (and possibly prognostic) significance might eventually make treatment of GVHD pre-emptive rather than prophylactic. Cellular component therapies—such as regulatory T cells that have been expanded ex vivo—will also enter clinical trials in the near future, but the extensive infrastructure needed for such approaches will probably restrict their use initially to large academic centres, intensifying need for close communication between transplant specialists and referring haematologists.

Conflict of interest statement
We declare that we have no conflict of interest.

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References


Seminar


