Oral Diseases (2007) 13, 519–529. doi:10.1111/j.1601-0825.2007.01412.x

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INVITED REVIEW

Mucosal graft-*vs*-host disease

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Graft-*vs*-host disease (GVHD) is a serious complication of hematopoietic stem cell transplantation (HSCT). Indications for HSCT have greatly expanded, and more patients are undergoing HSCT today than ever before. In addition, the options for immunosuppressive therapy for both prevention and treatment of GVHD have also expanded. These changes have in turn altered the landscape of this disease. We have reviewed the current literature on this subject and presented an update on this disease with a particular emphasis on mucosal manifestations.
Oral Diseases (2007) **13**, 519–529

Keywords: mucosa; inflammatory disease; stem cell transplantation

Hematopoietic stem cell transplantation

Bone marrow transplantation has been in existence for half a century. The first allogeneic hematopoietic stem cell transplantation (HSCT) was performed in 1957 to treat end-stage leukemia (Thomas *et al*, 1957). The discovery of human leukocyte antigens (HLA) in the same year, shed new light on the role of matching donor to recipient to avoid graft rejection and other complications such as graft-*vs*-host disease (GVHD) (Dausset, 1957). In those early days, HSCT was mainly indicated in patients with leukemias or severe combined immunodeficiency disorders. Since then, the indications for HSCT have greatly expanded (Table 1). The variety of stem cell sources has increased as well. In the past, stem cells were harvested from the bone marrow of a related donor, preferably an HLA-matched sibling. Nowadays, stem cells are either autologous or allogeneic, and can be derived from bone marrow, peripheral blood, banked cryopreserved umbilical cord blood, or fetal liver blood. In addition, the donor no longer needs to be related, but HLA-matching is still strongly preferred. The source of stem cells is an important factor in determining a patient's risk for GVHD.

In order to eradicate the host's diseased cells and prevent graft rejection, conditioning therapy is given before transplantation. One commonly used myeloablative regimen is cyclophosphamide administration followed by total-body irradiation (TBI) (Clift *et al*, 1998, 1999). An alternative regimen is busulfan used in conjunction with cyclophosphamide, without TBI (Ringden *et al*, 1999). Other agents that have been used for conditioning, with or without TBI include antithymocyte globulin (ATG), fludarabine and melphalan (Morris *et al*, 2004; Rzepecki *et al*, 2006). When used without TBI, these are considered reduced-intensity conditioning therapies, which in turn reduce transplant-related morbidity and mortality. Conditioning therapy is toxic in itself, and in determining the intensity of therapy used, the indication for HSCT is considered. In the case of malignant disorders, eradication should be aggressive; whereas for non-malignant indications a less intense therapy that does not cause myeloablation is adequate. After transplantation, immunosuppression is needed to prevent GVHD. Standard immunosuppressive therapy includes corticosteroids, cyclosporin and methotrexate alone or in combination. More recently, other agents have also been used with increasing success, including intravenous immunoglobulin, tacrolimus, sirolimus, and biologic agents such as alemtuzumab (anti-CD52) directed against lymphocytes (Morris *et al*, 2004; Rzepecki *et al*, 2006).

Background on GVHD

Despite advances in post-transplantation immunosuppression, GVHD remains a serious complication of HSCT. Although GVHD is classically associated with HSCT, it can also occur after solid organ transplantation, most frequently after small bowel and liver transplants (Key *et al*, 2004). The graft-*vs*-host reaction was described by Billingham (1966) to be caused by a population of graft-derived sensitized lymphoid cells attacking the host from within. In the setting of GVHD, the transplanted tissue contains immunologically competent cells that the host is incapable of rejecting. These cells are exposed to antigens that were not present in the

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Received 15 June 2007; accepted 18 June 2007

Table 1 Indicationsfor SCT

Leukemias
Acute myeloid leukemia
Acute lymphoblastic leukemia
Chronic myeloid leukemia
Myelodysplastic syndrome
Chronic lymphocytic leukemia
Lymphoproliferative disorders
Myeloma
Hodgkin's lymphoma
Non-Hodgkin's lymphoma
Solid tumors
Neuroblastoma
Glioma
Soft-tissue sarcoma
Germinal tumors
Breast cancer
Ewing
Lung cancer
Ovarian cancer
Renal cancer
Melanoma
Colon cancer
Other solid tumors
Non-malignant disorders
Severe aplastic anemia and Fanconi
Thalassemia
SCID
Inborn errors
Auto-immune diseases

transplant donor, and are thus recognized as foreign. The effector cells in the GVHD reaction are donor T cells, which differentiate self from foreign using the major histocompatibility complex (MHC) of the donor (Hexner, 2006). HLAs expressed on the cell surfaces of the recipient cells are the gene products of the recipient MHC. These foreign HLAs activate allogeneic T cells, reinforcing the importance of careful HLA matching when choosing an appropriate donor. However, even when host–donor pairs are HLA-identical, GVHD can be induced by minor histocompatibility antigens (mHag) within the host. The genes for these minor antigens are located outside of the MHC.

Graft-*vs*-host disease can be classified as either acute or chronic: acute occurring within the first 100 days of post-transplant, and chronic occurring thereafter. Although the general pathophysiology of both reactions is similar, the details of each are different. In acute GVHD, several factors contribute to the disease, beginning with conditioning therapy. Chemoradiation activates host antigen-presenting cells, which in turn stimulate proliferation of donor T cells. In addition, damage to the gastrointestinal (GI) tract because of TBI can release microbial lipopolysaccharides (LPS) into the systemic circulation, further stimulating donor T cells (Ferrara and Reddy, 2006). These activated T cells secrete cytokines including interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, a process known as cytokine storm. This complex and synergistic interaction between both innate and adaptive immune responses ultimately leads to the acute form of GVHD.

In chronic GVHD (cGVHD), the phenotype and origin of the alloreactive T cells are more ambiguous. It is assumed that aberrant thymopoeisis on the part of the host results in retention of autoreactive T-cell clones. Murine models of cGVHD show involution of thymic epithelium, depletion of lymphocytes, and loss of thymic function, implicating thymic injury as a major player in the development of cGVHD (Krenger *et al*, 2000; KM, 2004). Impaired ability in the host's T-regulatory cells (CD4+ and CD25+) to suppress the effector cells during host immune reconstitution contributes to the development of GVHD (Zorn, 2006). Similarities between cGVHD and autoimmune diseases such as autoantibody formation have been observed. However, the role of autoantibodies and B cells in the pathogenesis of cGVHD is still being investigated. A rat model developed by Beschorner *et al* demonstrated impaired cellular immune response and reduction of antibody response in rats with cGVHD. Antinuclear antibody levels in the diseased rats were also elevated, further suggesting that humoral immunity plays a pathologic role in cGVHD (Tutschka *et al*, 1982). A murine model of cGVHD also supports this notion by showing impaired elimination of autoreactive host B cells in mice with cGVHD (Gleichmann *et al*, 1984).

Apart from the diagnoses of acute or chronic GVHD, there is another clinical entity post-HSCT known as engraftment syndrome, sometimes referred to as hyperacute GVHD. Strictly speaking, this is not similar to the GVHD reaction occurring in autologous recipients. It is characterized by fever, erythematous skin lesions, and pulmonary edema. These are a result of dysregulated cytokine production that occurs immediately after engraftment (Deeg and Antin, 2006).

Graft-*vs*-host disease is a clinical diagnosis, and the manifestations of acute *vs* chronic disease are different. Acute GVHD is characterized by fever, skin eruptions, and intestinal and liver dysfunction. It is important to note that many of these symptoms may also be a result of the conditioning regimen itself, rather than a manifestation of GVHD. Clinical skin findings include a maculopapular, erythematous exanthema often involving the palms and soles. Typically, mucosal (oral, conjunctival, and vaginal) lesions are not symptoms of acute GVHD; however, mucositis that does not heal with hematologic recovery may be a sign of GVHD. Gastrointestinal involvement usually presents as nausea, vomiting, anorexia, pain, or diarrhea. Infectious causes of diarrhea should always be ruled out, as the host is in an immunocompromised state following transplantation. Biopsy of the intestinal mucosa in GVHD shows ulcerations, crypt destruction, crypt cell apoptosis and flattening of the villous architecture (Holmberg *et al*, 2006). Liver involvement is usually evidenced by as elevated bilirubin levels. Often, alkaline phosphatase levels are also elevated, but transaminase levels are less frequently affected. Clinically, there may be jaundice, but ascites and weight gain are rare. Once again, infectious causes and drug toxicity must be ruled out, and liver biopsy used to substantiate the diagnosis. Characteristic hepatic biopsy findings are segmental disruption of the small bile ducts, cellular degeneration,

and bile duct epithelium atypia, with or without cholestasis (Deeg and Antin, 2006).

While cGVHD also affects the skin, the GI tract, and liver, the manifestations are somewhat different from those of acute GVHD. In addition, chronic disease has pulmonary, hematologic, and musculoskeletal manifestations and affects oral, ocular and vaginal mucosa. Dermal lesions of cGVHD may resemble lichen planus. Histopathology demonstrates epidermal atrophy and dense focal dermal fibrosis in the absence of significant inflammation (Farmer, 1986). Some patients may present with a rash similar to that of acute GVHD, with rapid presentation, albeit delayed with respect to the time of transplant. Others may have an insidious onset of mottled hypo- and hyper-pigmentation, perifollicular papules, papulosquamous plaques, and eventual sclerodermatous changes (Figure 1). Alopecia and nail loss are common. Hepatic cGVHD presents mainly as cholestasis and hyperbilirubinemia. Lung involvement presents with a picture that clinically resembles chronic obstructive pulmonary disease (Horwitz and Sullivan, 2006). Histologically, it similar to obliterative bronchiolitis. Pulmonary involvement in cGVHD is a poor prognostic factor, with 5-year survival rates of only 10% (Dudek *et al*, 2003). Musculoskeletal involvement is primarily fascial, but myositis and arthritis can also occur. Fasciitis can range in severity from mild joint stiffness to extreme range of motion impairment and contractures (Filipovich *et al*, 2005). Mucosal manifestations of cGVHD are the focus of this review, and will be discussed separately.

Chronic GVHD is further characterized by a scoring system developed by the National Institutes of Health (NIH) (Filipovich *et al*, 2005). This scoring system is based on the extent of organ involvement, and is used to determine a patient's need for systemic treatment. Scoring ranges from 0 to 3 in any given system, including skin, mouth, eyes, GI tract, musculoskeletal system and genital tract. The scores of 0, 1, 2, 3 are assigned to definitions of no symptoms, mild symptoms, moderate symptoms and severe symptoms, respectively. Globally, the scoring system takes into consideration the number of organ systems involved and the severity in each individual system. Global scoring is not given a numeric value, but rather is simply graded as mild, moderate or severe. Mild cGVHD is defined as the involvement of one to two organs, with a maximum score of 1 in all affected organs. Moderate disease is defined as at least one organ involved with a score of 2, or three or more organ sites with a score of 1. Severe disease is defined as a score of 3 in any one organ, or a lung score of 2 or greater. Mild disease can usually be treated with local therapy (e.g. topical steroids), but any disease that is considered moderate or severe should be treated with systemic therapy, which will be discussed later.

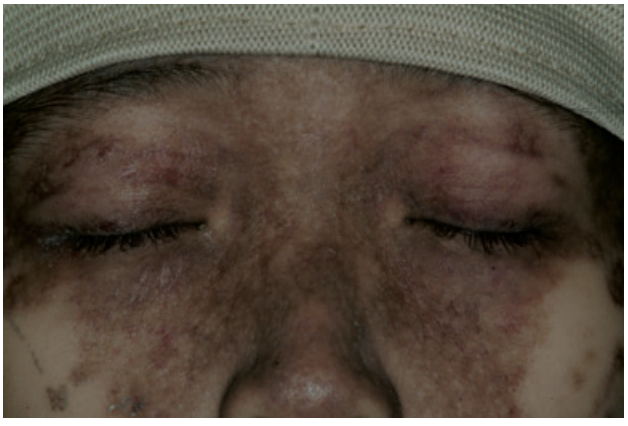

Figure 1 Cutaneous graft-*vs*-host disease (GVHD)

The reported risk of developing GVHD after HSCT is varied, and the results of several epidemiological studies are listed in Table 2. Several factors have a predictive value when determining a patient's risk for GVHD. As one would expect, increased HLA disparity is a principal risk factor for the development of GVHD (Sullivan *et al*, 1991). Related to this, female donors in male recipients cause greater GVHD, presumably because of antigens encoded on the Y chromosome. Advanced recipient age is also associated with higher risk of acute GVHD (Wagner *et al*, 2000; Remberger *et al*, 2002). This may be due to the enhanced allo-stimulatory activity of the APC cells of older patients compared to younger patients (Ordemann *et al*, 2002). A higher intensity conditioning regimen is also a positive risk factor for development of acute GVHD. These conditioning regimens act to enhance chances of developing acute GVHD in two ways. First, they liberate microbial antigens into the systemic circulation, which trigger the innate immune response. Secondly, irradiation damages host tissues, thereby inducing secretion of inflammatory cytokines. These responses are synergistic with the adaptive response of T cells in acute GVHD (Ferrara and Reddy, 2006). It should be noted that reducing the intensity of conditioning therapy may also reduce the likelihood of successful engraftment. Finally, the source of donor cells is an important risk factor. Several studies have reported a lower incidence of GVHD associated with cord blood and bone marrow when compared with peripheral blood (Vigorito *et al*, 1998; Blaise *et al*, 2000; Heldal *et al*, 2000; Cutler *et al*, 2001; Korbling and Anderlini, 2001). Mature T cells in the peripheral blood were shown to be more potent in inducing GVHD than those from bone marrow (Zeng *et al*, 1999). It is postulated that contamination of bone marrow with peripheral blood may contribute to the development of GVHD. Pre-treatment of donor tissue to deplete T cells prior to transplantation is an effective form of GVHD prophylaxis; however, this also predisposes the host to potentially fatal opportunistic infections (Prentice *et al*, 1984; Ringden *et al*, 1991).

Chronic GVHD has its own set of risk factors, in addition to those listed above. Acute GVHD is in itself a risk factor for the development of chronic disease (Atkinson *et al*, 1990). Finally, patients with a diagnosis of chronic myelogenous leukemia (CML) as an indication for HSCT are at higher risk for cGVHD when compared with other diagnoses. The role of CML in the

Table 2 Occurrence of mucosal GVHH after BMT

References	*Description of paper*	*Total patients studied*	*Type of GVHD*	*Ocular (%)*	*Oral (%)*	*Esophageal (%)*	*Vaginal (%)*
Glucksberg *et al* (1974)	GVHD in HLA-matched donors	61					
Sullivan, *et al* (1981)	cGVHD after allogeneic BMT	175	Chronic	24.6	26.3	9.7	
Franklin *et al* (1983)	Ocular manifestations of GVHD after BMT	27	Ocular	44.4			
Mettinger *et al* (1991)	Ocular manifestations of GVHD after BMT	53	Ocular	41.5			
Tichelli, *et al* (1996)	Late-onset KCS after bone marrow transplantation	248	Ocular	13.3			
Kerty *et al* (1999)	Ocular findings in allogeneic SCT	130	Ocular	22.3			
Mohty *et al* (2002)	cGVHD cumulative incidence in PBSC v. BMT	48 (PBSC)	Ocular/oral/vaginal	45.8	60.4		6.3
Mohty, *et al* (2002)		53 (BMT)	Ocular/oral/vaginal	13.2	13.2		3.8
Bradfield *et al* (2005)	Ocular complications in children after BMT	74	Ocular	4.1			
Leite *et al* (2006)	Dry eye after progenitor stem cell transplant	124	Ocular/acute/chronic	8.9			
Balaram *et al* (2006)	Chronic ocular surface disease after allogeneic BMT	62	Ocular/acute/chronic	79.0			

development of GVHD is controversial, but one hypothesis is that it is due to the higher rate of splenectomy in CML patients (Carlens *et al*, 1998).

First-line therapy for both acute and chronic GVHD is still systemic corticosteroids and cyclosporin. These agents are used for both prophylaxis and treatment of GVHD in doses that range from 1 to 5 mg/kg/day for cyclosporin and 2 to 20 mg/kg/day for methylprednisolone (Van Lint *et al*, 1998; Michallet *et al*, 1999). For refractory cases, agents similar to those used for conditioning therapy have been tried with some success, including tacrolimus, sirolimus (Johnston *et al*, 2005), azathioprine (Sullivan *et al*, 1988), low-dose TBI (Socie *et al*, 1990), thalidomide (Vogelsang *et al*, 1992), and mycophenolate mofetil (MMF) (Krejci *et al*, 2005; Lopez *et al*, 2005; Takami *et al*, 2006). For acute GVHD, which is thought to be more T-cell-mediated, ATG and monoclonal antibodies directed against T cells such as daclizumab have been used, (anti-IL2) (Bruner and Farag, 2003; Lee *et al*, 2004; Rodriguez *et al*, 2005; Wolff *et al*, 2005; Teachey *et al*, 2006). Both T and B cells are involved in cGVHD, and anti-B-cell biologics such as rituximab (anti-CD20) have also been used as treatment (Cutler *et al*, 2006). Recently, palifermin, a recombinant human keratinocyte growth factor, was subjected to phase I/II trials for prevention of acute GVHD, but was found to have no significant effect on acute GVHD or survival (Blazar *et al*, 2006). For cases refractory to the treatments listed above, extracorporeal photochemotherapy (ECP) was shown to be effective (Komanduri *et al*, 2006). In ECP, autologous peripheral blood mononuclear cells are collected by apheresis, and photosensitized with 8-methoxypsoralen (8-MOX). These cells are then irradiated with ultraviolet light A (UVA) light, and infused back into the patient. One series showed complete skin and oral mucosal resolution in 80% and 100% of steroid-refractory patients treated with ECP, respectively (Greinix *et al*, 1998). Finally, the use of mesenchymal stem cells (MSC) in steroid-refractory cGVHD also shows promise as a therapy (Ringden *et al*, 2006).

Mucosal GVHD

The remainder of this review will focus on GVHD from a mucosal perspective. GVHD has the potential to affect all mucosal surfaces, including ocular, oral, vaginal, and gastrointestinal. A summary of the mucosal manifestations of GVHD can be found in Table 3. In general, the landscape of this disease is changing with the advent of a wider array of immunosuppressive therapies. In reviewing the literature, the reported incidence of mucosal involvement appears to be trending down. For example, multiple studies prior to 1985 report ocular manifestations of acute GVHD to be as high as 60–70% (Hirst *et al*, 1983; Franklin *et al*, 1983) A study published in 1999 showed ocular involvement in acute GVHD to be only 10% (Kerty *et al*, 1999). Leite *et al* (2006) found only 32% of 124 patients to have any dry eye manifestations after HSCT. Furthermore, they observed no chronic severe conjunctivitis in the same group of 124 patients. Variation in the study populations of these reports, such as conditioning therapy, age, and source of stem cells, should be considered when interpreting these studies.

Ocular disease

Ocular GVHD affects mainly the conjunctiva, cornea, and lacrimal gland. Scleritis had been reported as the initial manifestation of cGVHD, but this is not common (Kim *et al*, 2002). Rarely, retinal pathology can be seen after HSCT, including central serous retinopathy, retinal pigmented epitheliopathy and retinal microvasculopathy; some authors have questioned whether these manifestations are due to GVHD or are side effects of immunosuppressive therapy (Fawzi and Cunningham, 2001; Strouthidis *et al*, 2003; Cheung *et al*, 2004;

Table 3 Manifestationsof mucosal graft-*vs*-host disease

	Acute	*Chronic*
Ocular surface	Conjunctivitis	Aqueous tear deficiency (dry eye)
	Hemorrhagic conjunctivitis	Superficial punctate keratitis
	Pseudomembranous conjunctivitis	Filamentary keratitis
	Serosanguinous discharge	Corneal ulceration (infectious and sterile)
	Chemosis	Corneal perforation
	Sloughing of corneal epithelium	Canalicular and nasolacrimal duct obstruction
	Cicatrizing conjunctivitis	Persistent corneal epithelial defect
		Corneal neovascularization
		Calcareous degeneration of cornea
		Keratinization
		Superior limbic keratoconjunctivitis
		Conjunctival fibrosis
		Episcleritis
		Foreshortening of fornices
		Cicatricial entropion and lagophthalmos
		Symblepharon
Oral mucosa	Non-healing mucositis	Xerostomia (dry mouth)
		Mucosal atrophy or ulceration
		Mucosal erythema
		Sclerodermatization / lichenification
		Hyperkeratosis
		Pyogenic granuloma
Gastrointestinal	Elevated liver function tests	Achalasia
	Abdominal cramping	Esophageal strictures
	Nausea	Dysphagia
	Emesis	
	Diarrhea	
Genitourinary	None reported	Vaginal dryness
		Dyspareunia
		Ulcerated or excoriated mucosa
		Vaginal strictures

Kawase *et al*, 2005). Vitritis has also been reported as a manifestation of ocular GVHD, but this is exceedingly rare (Sheidow *et al*, 2004). Conjunctival involvement ranges from mild hyperemia to pseudomembranous and cicatrizing conjunctivitis, similar to that seen in ocular cicatricial pemphigoid (Saito *et al*, 2002; Karwacka *et al*, 2006). The conjunctivitis of acute GVHD differs from that of cGVHD, in that it is more often hemorrhagic and ulcerative. The hemorrhagic stage is followed by an exudative stage, in which there is a sterile, purulent discharge of polymorphonuclear leukocytes with pseudomembrane formation (Jack *et al*, 1983). This, in turn, is followed by ulceration, and finally, scarring of the conjunctiva. Kim *et al* (2005) proposed the careful debridement of pseudomembranes and topical corticosteroid administration to limit cicatrization. Aside from the conjunctivitis described above, other anterior segment manifestations of GVHD have been described, including superior limbic keratoconjunctivitis and episcleritis. While conjunctival involvement in acute GVHD is relatively uncommon it remains a poor prognostic factor, and is a marker for severe systemic involvement (Johnson and Jabs, 1997).

Conjunctival involvement in GVHD can be staged based on extent of involvement. Staging may be more relevant in clinical trials than in clinical practice, as it serves as an outcome measure for studies. Two conjunctival staging schemes have been proposed in the existing literature. Jabs *et al* (1989) described a grading scheme most appropriate to describe acute ocular GVHD: stage I as hyperemia; stage II as hyperemia with serosanguinous chemosis; stage III as pseudomembranous conjunctivitis; and stage IV as pseudomembranous conjunctivitis with corneal epithelial sloughing. Robinson *et al* (2004) developed a grading system, which is useful to assess the extent of chronic ocular GVHD: grade 1, hyperemia; grade 2, palpebral conjunctival fibrovascular changes involving $<25\%$ of the palpebral conjunctiva, with or without epithelial sloughing; grade 3, palpebral conjunctival fibrovascular changes of the palpebral conjunctiva involving 25–75% of the total surface area; and grade 4, involvement of $>75\%$ of total surface area with or without cicatricial entropion (Figure 2).

Conjunctival biopsies in acute GVHD demonstrate a substantia propria inflammatory cell infiltrate composed predominantly of CD4+ helper T lymphocytes rather than CD8+ suppressor/cytotoxic lymphocytes (Boozalis *et al*, 1987). In the ulcerative stages, histology of the tarsal conjunctiva shows a plasmocytic infiltration of the subepithelium and loss of epithelial integrity. Conjunctiva in cGVHD shows increased expression of the adhesion molecule Intercellular Adhesion Molecule (ICAM)-1 and a decreased number of goblet cells (Aronni *et al*, 2006).

Corneal involvement is more common and pronounced in chronic than in acute GVHD. In acute GVHD, there may be filamentary keratitis, or corneal

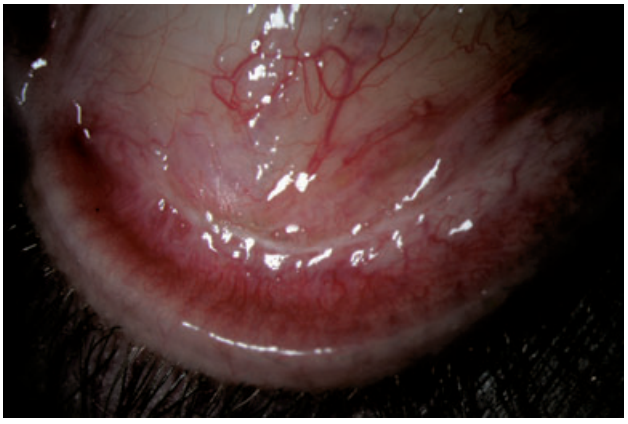

Figure 2 Grade 3 conjunctival graft-*vs*-host disease (GVHD)

sloughing, but this is often secondary to lacrimal gland or conjunctival inflammation described above, rather than a direct effect of T-cell *vs* tissue reaction (Jack *et al*, 1983). Rarely, calcareous degeneration of the cornea with perforation may occur (Yeh *et al*, 2006). Chronic ocular GVHD most commonly manifests as moderate to severe keratoconjunctivitis sicca (KC sicca) (Figure 3). The dry eye of GVHD is often severe, and can lead to filamentary keratitis, corneal ulceration, scarring, melt, and ultimately perforation if not adequately treated (Yoshida *et al*, 2006). The GVHD reaction in the lacrimal gland results in acinar tissue destruction and permanent tear dysfunction. Infiltratins CD4+ and CD8+ T cells exert cytotoxic effects on the periductal epithelial cells, impairing lacrimal gland exocrine function (Ogawa *et al*, 2003a,b; Hassan *et al*, 2005). A murine model studied by Hassan *et al* (2005) demonstrates this histopathologically. Further studies have demonstrated periodic acid-Schiff (PAS)-positive material clogging and distending lacrimal gland ducta, further impairing the ability to secrete tears (Jabs *et al*, 1983). In addition to decreased tear production, cicatricial lagophthalmos or ectropion lead to poor tear film distribution, exacerbating the ocular surface disease (Jabs *et al*, 1989).

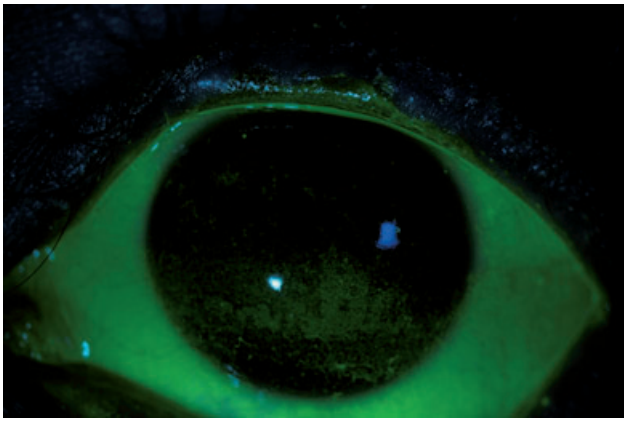

Figure 3 Punctate keratitis in graft-*vs*-host disease (GVHD)

Treatment of ocular disease is geared toward rigorous lubrication, and prevention of corneal and conjunctival scarring. Early aggressive use of topical corticosteroids has been proposed; however, no randomized, controlled trials or limited clinical studies have been performed to demonstrate their safety and efficacy in a large patient population (Robinson *et al*, 2004). Topical cyclosporin A, retinoic acid, and autologous serum tears have also been used with some success to treat established ocular GVHD in small-scale studies (Murphy *et al*, 1996; Rocha *et al*, 2000; Ogawa *et al*, 2003a,b; Leite *et al*, 2006). However, often topical therapy alone is insufficient, and surgical intervention such as permanent punctal occlusion, tarsorrhaphy, or amniotic membrane graft is necessary (Peris-Martinez *et al*, 2001). If corneal ulceration or perforation does occur, lamellar or penetrating keratoplasty can be performed in an attempt to salvage the eye, but success is limited because of frequent recurrences and severe dryness. There are also more novel therapies that are currently under investigation. Systemic use of tacrolimus is also reported to be effective against the KC sicca of cGVHD (Ogawa *et al*, 2001; Ahmad *et al*, 2002; Aoki *et al*, 2005). Evaluation of an episcleral cyclosporin implant in animal models shows promise as a novel therapy for ocular surface disease and an ongoing clinical trial is evaluating this novel therapy in GVHD at NIH.

Oral disease

Oral mucosal disease is a common manifestation of cGVHD. One report found it to be the most common manifestation of cGVHD in peripheral blood HSCT, and the second most common manifestation in bone marrow-derived HSCT, occurring in as many as 60% of patients receiving peripheral blood HSCT (Mohty *et al*, 2002). Symptoms of oral involvement are pain, alteration in taste, and dry mouth. Clinically, lesions sometimes appear as lichenoid changes, mucosal atrophy or ulceration; however, symptoms may occur in the absence of noticeable lesions. Secondarily, dry mouth can lead to dental caries and oral superinfection. Oral discomfort is also often associated with anorexia and weight loss.

Dry mouth is a direct result of salivary gland exocrine dysfunction. Salivary gland dysfunction is also associated with radiation and chemotherapeutic agents used in conditioning therapies. Therefore, salivary gland biopsy or lip biopsy containing both mucosal and minor salivary gland tissue is sometimes used to diagnose GVHD (Nakhleh *et al*, 1989). Histopathologically, the salivary glands in cGVHD have features that resemble the lacrimal glands as previously described. Oral histopathology is similar to that of Sjogren's syndrome, except that there are fewer lymphocytes which are not organized in foci (Lamey *et al*, 2004). In addition to decreased production of saliva, cGVHD also changes the composition of saliva. Patients who develop cGVHD have a significant decrease in their salivary proteins including Secretory Immunoglobin A (sIgA), as well as a decrease in salivary antioxidants. This change

results in a reduction in the protective capacity of the saliva, further exacerbating oral mucositis in cGVHD (Nagler *et al*, 2006). Oral epithelium shows destruction of the basal and suprabasilar cells, with interspersed areas of hyperkeratosis similar to those seen in the epidermis of GVHD patients. This sclerodermatization can even result in restriction in mouth opening, exacerbating decreased food intake and weight loss.

Oral mucositis associated with cGVHD can respond fairly well to systemic therapy. Of the systemic therapies already discussed, several have been evaluated specifically for oral mucosal disease and have been shown to have a positive effect. These therapies include rituximab, sirolimus, ECP, hydroxychoroquine, thalidomide, pentostatin, MMF, and clofazimine (Canninga-van Dijk *et al*, 2004). Of note, although methotrexate has been shown to be effective in oral cGVHD (Giaccone *et al*, 2005; Huang *et al*, 2005), it is also known to be a mucotoxic agent. Often, systemic therapies are combined with local therapy, which consists of immunomodulatory drugs in the form of a mouth rinse or gel. Agents that are available in this form include cyclophosphamide, dexamethasone, azathioprine, budesonide, and tacrolimus. All have been evaluated in small-scale studies and with some efficacy (Imanguli *et al*, 2006). Finally, the use of local phototherapy has also been evaluated in several small-scale studies. Oral lesions refractory to systemic therapy were exposed to UVA after administration of 8-methoxypsoralen (PUVA) and showed some degree of improvement (Atkinson *et al*, 1986; Elad *et al*, 1999). One should be prudent in the use of PUVA, as it also increases the patient's risk of oral squamous cell and basal cell carcinomas. In addition to these therapies which target the underlying cause of cGVHD, additional supportive therapies for oral cGVHD include antibacterial mouthwashes, topical analgesics (e.g. lidocaine), artificial saliva and salivary stimulants. Meticulous oral hygiene is also important in these patients to prevent secondary infections.

Gynecologic disease

The gynecologic mucosal manifestations of GVHD are less well characterized than those of ocular and oral mucosa. Vaginal involvement in GVHD was first described in 1982 as sclerosing vaginitis and stricture formation (Corson *et al*, 1982). Symptoms include dryness, irritation, dyspareunia, and postcoital bleeding. It is unclear whether the signs and symptoms of vaginal mucosal involvement are due to hypoestrogenism from premature ovarian failure secondary to conditioning therapies, or to the GVHD itself (Spiryda *et al*, 2003). Regardless, there is a fair prevalence of vulvar and vaginal involvement in women who have undergone HSCT. One retrospective study showed genital lesions consistent with GVHD in 24.9% of patients after HSCT (Spinelli *et al*, 2003). Histopathology of vagina- or vulvar mucosa-affected lesions demonstrates foci of chronic and acute inflammation, with lichenoid or spongiotic dermatitis and hyperkeratosis. These findings are similar to skin findings in dermal cGVHD. This evidence supports the theory that the GVHD reaction is involved in the pathology, as these changes cannot be explained solely on the basis of hypoestrogenism. However, in some series, vaginal and vulvar symptoms did not correlate with the severity of GVHD found in other organ systems (Spiryda *et al*, 2003). Treatment of gynecologic cGVHD includes systemic and topical estrogen administration. In cases where stricture and scarring prevent vaginal intercourse, surgery can be performed to reconstruct the vaginal canal. The role of immunomodulation in gynecologic cGVHD is not well defined. However, one series reported success with use of topical cyclosporin in mild to moderate disease (Spiryda *et al*, 2003).

Gastrointestinal disease

Intestinal involvement in cGVHD is uncommon. Clinically, GI cGVHD can symptomatically resemble acute disease, presenting as nausea, diarrhea, and/or vomiting. In fact, some postulate that GI cGVHD may represent persistent acute GVHD (Akpek *et al*, 2003). The mucosal lesions in the lower GI tract of cGVHD are essentially identical to those in acute GVHD, described earlier. However, there are some manifestations of chronic GI disease that are not seen in the acute phase, especially those of the upper GI tract. Esophageal fibrosis results in poor motility, and in more severe cases, strictures and webs (McDonald *et al*, 1981). The resulting dysphagia, when coupled with the vomiting, diarrhea, and malabsorption of GI GVHD often results in anorexia and significant weight loss. Esophageal involvement is probably the least common of all mucosal cGVHD manifestations, but there is little epidemiologic data. GI GVHD usually responds well to corticosteroid therapy administered either intravenously, or orally in a poorly absorbed form, e.g. beclomethasone (Iyer *et al*, 2005).

Ancillary therapy

Graft-*vs*-host disease potentially affects multiple organ systems, including all mucosal surfaces. As described, mucosal clinical manifestations ultimately result from insufficient moisture and lubrication. In the case of oral and ocular disease, the pathology exists primarily in the exocrine function of salivary and lacrimal glands respectively. Esophageal strictures result from both an insufficiency of lubrication from decreased salivary secretions, and atrophy of the esophageal mucosal surface from the GVHD reaction itself. Similarly, vaginal strictures result from decreased vaginal secretions because of hypoestrogenism and mucosal atrophy from GVHD. In the treatment of mucosal GVHD, ancillary and supportive therapy is essential whether or not systemic immunosuppression is indicated. This ancillary therapy should be geared toward providing adequate lubrication to all surfaces involved and preventing secondary infection. Recently, a report of the NIH Consensus Development project on ancillary therapy and supportive care made recommendations for

such therapy, and many of these have already been mentioned. Treatments including artificial saliva, artificial tears, vaginal gels, anti-microbial therapy, and meticulous hygiene of all involved areas are all important in successfully managing mucosal disease (Couriel *et al*, 2006).

Graft-*vs*-host disease affects many aspects of the HSCT patient's quality of life, and ancillary therapy should take into consideration the whole patient, and not just the particular system or systems affected. Depression and fatigue frequently accompany this disease and counseling and pharmaceutical therapy may be needed. Nutritional support is also extremely important, as many of these patients suffer from anorexia for one reason or another. Finally, systemic immunosuppressive therapy itself is fraught with adverse side effects including, but not limited to susceptibility to infection, loss of bone density (in the case of chronic steroid use), hematopoietic abnormalities, compromised liver function and GI disturbances. Patients must be carefully monitored for all of these and treated accordingly.

Conclusion

Graft-*vs*-host disease remains a significant and serious problem after HSCT. An expanding list of indications for HSCT and immunosuppression options post-HSCT means more patients are undergoing this procedure and surviving. Immunosuppression plays a key role in the prevention and treatment of GVHD, but a balance must be struck between appropriately immunosuppressing the patient, yet allowing for successful engraftment and desired graft *vs* tumor effect. Ongoing research will hopefully shed new light on questions involving pathophysiology of the disease, especially in its chronic form. Efforts are also underway to investigate the utility of routine prophylaxis for mucosal disease (i.e. use of topical immunosuppressives). As we learn more about this disease, we hope to shift its clinical spectrum toward the milder side, and develop more effective prevention strategies.

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