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Novel Scoring Criteria for the Evaluation of Ocular Graft-versus-Host Disease in a Preclinical Allogeneic Hematopoietic Stem Cell Transplantation Animal Model

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A B S T R A C T

Ocular complications occur after transplantation in 60% to 90% of chronic graft-versus-host disease (GVHD) patients and significantly impair vision-related quality of life. Ocular surface inflammation and dry eye disease are the most common manifestations of ocular GVHD. Ocular GVHD can be viewed as an excellent preclinical model that can be studied to understand the immune pathogenesis of this common and debilitating disease. A limitation of this is that only a few experimental models mimic the ocular complications after hematopoietic stem cell transplantation (HSCT) and have focused on the acute GVHD process. To address this issue, we used a preclinical animal model developed by our group where ocular involvement was preceded by systemic GVHD to gain insight regarding the contributing immune mechanisms. Employing this "matched unrelated donor" model enabled the development of clinical scoring criteria, which readily identified different degrees of ocular pathology at both the ocular surface and adnexa, dependent on the level of conditioning before HSCT. As far as we are aware, we report for the first time that these clinical and immune responses occur not only on the ocular surface, but they also heavily involve the lid margin region. In total, the present study reports a preclinical scoring model that can be applied to animal models as investigators look to further explore GVHD's immunologic effects at the level of the ocular surface and eyelid adnexa compartments. We speculate that future studies will use this clinical scoring index in combination with what is recognized histologically and correlated with serum biomarkers identified in chronic/ocular GVHD.

INTRODUCTION

Graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) is a multiorgan disorder stemming from an immunological attack by donor allo-reactive T cells, resulting in damage to the liver, skin, gastrointestinal tract, hematopoietic tissues, and additional compartments during chronic disease, including the ocular surface of the eye [1]. Ocular complications occur after transplantation in 60% to 90% of chronic GVHD patients and significantly impair vision-related quality of life [2-5]. Ocular surface inflammation and dry eye disease are the most common manifestations of ocular GVHD and are hallmark findings in chronic GVHD [1,2]. Similar to other forms of dry eye syndrome related to inflammation, ocular GVHD can lead to loss of vision due to refractive changes as a result of the lacrimal film and, in severe cases, secondary to corneal ulceration/perforation [6]. Furthermore, it is known clinically that lid margin abnormalities contribute to ocular surface disease, often in the form of meibomian gland dysfunction [7], which is also a common manifestation of GVHD [8]. In contrast to other ocular surface disorders, in which there could be multiple pathways of disease, ocular GVHD disease is primarily immune mediated with a "time zero" initiation (transplantation), which allows more accurate monitoring to dissect the underlying immune pathogenesis [9,10].

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Therefore, ocular GVHD provides a useful model to test novel therapies for the prevention and treatment of dry eye.

Interestingly, there have been reports of ocular involvement actually preceding the diagnosis of clinical chronic systemic GVHD; regardless, early recognition of ocular pathology would enable more timely initiation of systemic and local therapies [11]. Although specific criteria exist for the diagnosis and assessment of systemic and ocular GVHD in humans [12], precise scoring criteria to evaluate ocular involvement in animal studies have not been established. The standardization of ocular manifestations of GVHD in preclinical animal models would not only assist in the evaluation of patient manifestations of ocular GVHD but also provide a method to uniformly communicate progression of disease and impact of interventional therapies.

Presently, few experimental models mimic the ocular complications after HSCT, and those almost exclusively focus on the acute GVHD process [13,14]. To address this issue, we generated a preclinical animal model in which ocular involvement was preceded by systemic GVHD to gain insight regarding the contributing immune mechanisms [15]. Our results demonstrated that after experimental MHC-matched minor histocompatibility–mismatched HSCT, ocular GVHD involves the presence of donor T cells in the cornea, as well as conjunctiva and lacrimal gland involvement, which lead to pathologic changes in the ocular compartment [15]. Notably, employing this MHC-matched, minor transplantation–antigen mismatched allogeneic "matched unrelated donor" (MUD) model has allowed us to develop clinical scoring criteria that readily identified different degrees of ocular pathology at both the ocular surface and adnexa, dependent on the level of conditioning before HSCT. In this study, we exploit our ability to monitor, in real time, the association of these clinical changes with the development of immune responses around the ocular adnexa to validate the role of inflammation in our scoring scale. To our knowledge, we report for the first time that these clinical and immune responses occur not only on the ocular surface, but that they also heavily involve the lid margin region.

METHODS

Animals

All animal studies were conducted according to protocols approved by the University of Miami animal care and use committee and in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL/6J (B6) ($H2^b$), C3H.SW ($H2^b$), B6.PL-Thy1a/CyJ (B6-Thy1.1), and enhanced green fluorescent protein (eGFP) B6 transgenic ($H2^b$) mice were initially obtained from Jackson Laboratory (Bar Harbor, ME) and maintained in the animal facilities at the University of Miami School of Medicine. All mice used in experiments were 8 to 10 weeks old, free from ocular surface and eyelid disease at baseline, and fed with a standard caloric diet for their age. The animals were routinely monitored before all procedures and until the end of the experiment.

HSCT

Mice were temporarily placed in a holding device to transport them for total body irradiation (TBI) with 7.5 or 10.5 Gy (n = 8) using a Gammacell 40 device (Best Theratronics Ltd., Ottawa Ontario, Canada) 3 to 4 hours before transplantation. All animals were provided antibiotic water from day -3 to day 14 after transplantation for prophylaxis against bacterial infection. Donor cells were obtained from unmanipulated mice differing from recipients at selected genetic loci. Donor B6 mice (H-2^b, Thy1.1) were euthanized by cervical dislocation, and lymph node tissue was harvested and processed as previously described [16,17]. Femurs and tibiae were removed from donor B6-eGFP$^+$ mice and bone marrow cells were flushed with cold RPMI. Donor marrow inoculum (TCD-BM) was prepared using anti-Thy-1.2 Miltenyi MACS magnetic beads (Miltenyl Biotec, Gaithersburg, MD) and negative selection to remove T cells, washed, and adjusted before transplantation to 5×10^6/mL. To prepare donor T cells, lymph node cells were incubated on anti–surface immunoglobulin-coated plastic dishes for 45 minutes at 4°C to remove B cells. Cell suspensions containing donor bone marrow and T cells were adjusted in serum-free RPMI to a concentration of 4.6×10^6/mL for intravenous (.5 mL) injection of 2.3×10^6 T cells/mouse.

Systemic GVHD Assessment

The immune phenotype of systemic GVHD was assessed by fluorescent-conjugated mAbs to analyze the CD4/CD8 ratio and B cell levels in peripheral blood. Clinical scoring was performed and recorded on all mice at baseline and through weeks 2 to 7 after transplantation.

Animals were monitored for established signs of GVHD by clinical assessment using a modified version of a standard scoring system previously described by Cooke et al. [18]. This system incorporates 7 clinical traits measuring the degree of systemic GVHD: posture, activity, weight loss, fur texture, skin integrity, degree of alopecia, and presence of diarrhea. Each clinical parameter was scored from 0 to 2, resulting in a total score that ranges from 0 to 14.

Clinical Evaluation of Ocular GVHD

Clinical photographs were obtained to evaluate clinical characteristics of ocular disease progression. Clinical components analogous to what is monitored in patients with ocular GVHD were used to develop the scoring system in mice after allogeneic HSCT. The rationale for the selection of these scoring criteria was based on clinical changes classically reported in the eyes of patients with chronic GVHD [3,19,20]. For the murine studies, at each time of analysis, individual animals were evaluated and graded from 0 to 4 for the clinical parameters related to both clinical manifestations, reflecting the spectrum from no involvement to severe manifestations in each anatomical domain (Table 1).

In Vivo Evaluation of Immunological Evaluation of Ocular GVHD

To correlate the clinical ocular findings and scoring system to in situ immunological responses, mouse recipients of eGFP$^+$-expressing cell populations were assessed at weekly time points using intravital fluorescent microscopy, allowing precise measurement of eGFP in the cornea and eyelid, which was quantified as mean green intensity (MGI) as described previously [21].

Table 1
Ocular Criteria and Scoring System Used To Assess Preclinical Model with Ocular GVHD

Total Ocular GVHD Score	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Clinical lid margin	Clear	Mild lid edema	Edema and partial lid closure	Edema, partial lid closure, skin swelling	Full lid closure
eGFP lid margin	Clear	Minimal eGFP infiltrate: upper/lower lid	eGFP infiltrate: upper/lower lid	eGFP infiltrate both lids and 0-1 mm skin involvement	eGFP infiltrate both lids and 1 mm skin involvement
MGI lid margin	0-25	25-50	50-75	75-100	>100
Clinical cornea	Clear	Epithelial haze	Diffuse keratopathy, pupil visualized	Confluent keratopathy, pupil not visualized	Ulceration
eGFP cornea	Clear	eGFP infiltrate: 25% cornea/limbus	eGFP infiltrate: 50% cornea/limbus	Diffuse eGFP infiltrate: 100% cornea	Confluent eGFP infiltrate: 100% cornea
MGI cornea	0-25	25-50	50-75	75-100	>100

Total ocular GVHD score incorporates all clinical, eGFP, and MGI scoring parameters encompassing both the lid margin scoring index and cornea scoring index to generate a total ocular GVHD score with a maximum value of 24. The level of scores obtained were compared with total systemic GVHD scores to monitor overall disease progression.