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Up-Regulation of α4β7 Integrin on Peripheral T Cell Subsets Correlates with the Development of Acute Intestinal Graft-versus-Host Disease following Allogeneic Stem Cell Transplantation

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Acute graft-versus-host disease (aGVHD) is a major complication after hematopoietic stem cell transplantation (HSCT). The pathophysiology of aGVHD involves priming of naïve donor T cells in host secondary lymphoid tissue, followed by migration of effector T cells to target organs. Mediators of lymphocyte trafficking are believed to play a significant role in this migration. In this retrospective case-controlled study, we analyzed the expression of α4β7 integrin and CCR9, 2 surface T cell molecules specific for intestinal trafficking, from blood samples collected previously from 59 patients after HSCT (20 without aGVHD, 20 with skin aGVHD, and 19 with intestinal aGVHD). All samples had been obtained before the onset of aGVHD symptoms (with 1 sample collected on the day of symptom onset). Analysis by flow cytometry demonstrated that α4β7 integrin was significantly increased on both naïve and memory T cells in patients who subsequently developed intestinal aGVHD, with the most significant differences observed in memory subsets. Immunohistochemical staining on rectal biopsy specimens from patients with intestinal aGVHD showed that expression of α4β7 integrin was concentrated on mononuclear cells in blood vessels within the intestinal mucosa. These results suggest that α4β7 integrin likely is involved in lymphocyte trafficking in intestinal aGVHD and may have potential clinical use as a correlative biomarker or as a target for the treatment and prophylaxis of intestinal aGVHD after HSCT.

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INTRODUCTION

Acute graft-versus-host-disease (aGVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT) [1]. It is caused by the recognition of host antigens by donor lymphocytes, with target organ damage mediated by activated donor effector T cells. With its large mucosal surface and extensive secondary lymphoid tissue, the gastrointestinal (GI) tract is not only a major target organ, but also a crucial amplifier of a systemic cytokine response in aGVHD [2]. Intestinal aGVHD is believed to be similar to other immunologic responses in that it has similar requirements for lymphocyte trafficking. Priming and maturation of naïve donor T cells that are targeted for the gut mucosa are believed to be mediated by activated host dendritic cells within gut-associated secondary lymphoid tissue (GALT), including Peyer's patches (PPs) of the small intestine, the appendix, and mesenteric lymph nodes [3]. Recent evidence has suggested that effector T cells acquire an intestinal homing phenotype through interactions with GALT dendritic cells in a process mediated by retinoic acid [4-8]. Once T cells have been educated in GALT through antigen engagement and costimulation, they continue to circulate through the bloodstream, but preferentially migrate to intestinal effector sites—the lamina propria and epithelium of the small and large intestine—as directed by their specific surface molecules [9,10]. This process of specific lymphocyte trafficking represents a potentially novel

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target for the monitoring, treatment, and prophylaxis of intestinal aGVHD.

Several classes of membrane surface molecules, including selectins, integrins, chemokine receptors, and pertussis-sensitive G-proteins, are involved in lymphocyte trafficking; all of these help ensure organ-specific localization of lymphocytes [11-13]. One specific molecule, α4β7 integrin, plays a crucial role in the recirculation of naïve T cells to intestinal secondary lymphoid tissue, as well as the selective trafficking of specific effector T cells into sites of intestinal inflammation [14-16]. The primary ligand for α4β7 integrin is mucosal addressin cell adhesion molecule 1 (MAdCAM-1), which is selectively expressed in the high endothelial venules and follicular dendritic cells of GALT, with specific up-regulation at sites of active inflammation [17-20]. Chemokines are thought to participate in this process through the activation of α4β7 integrin [21-23]. Although the precise chemokines in this process involved are unknown, the CCR9–CCL25 interaction has emerged as a good candidate [24,25]. CCL25 (TECK) has been shown to be expressed only in epithelial cells of the thymus and small intestine [26], and the vast majority of CCR9-expressing T cells in the peripheral blood express α4β7 integrin as well [27].

In the present study, we analyzed peripheral blood (PB) samples collected previously from 59 human patients after HSCT, to assess the involvement of α4β7 integrin and CCR9 in lymphocyte trafficking in intestinal aGVHD. We analyzed the expression of α4β7 integrin and CCR9 on specific T cell subsets by flow cytometry. α4β7 integrin was found to be significantly up-regulated on both naïve and memory T cell subsets in patients who subsequently developed intestinal aGVHD compared with those who developed primarily cutaneous aGVHD or did not develop aGVHD. Immunohistochemical staining on previously obtained rectal biopsy specimens in patients with intestinal aGVHD showed the presence of α4β7 integrin on inflammatory mononuclear cells aggregated within mucosal blood vessels. Our findings suggest that α4β7 integrin is up-regulated on peripheral blood T cells before the onset of symptomatic intestinal aGVHD, implying a role for this molecule in the pathophysiology of aGVHD.

METHODS

Patients and Samples

This was a retrospective case-controlled analysis using patient samples identified from the stem cell transplantation database at the Dana-Farber Cancer Institute (DFCI). The study design was approved by the Institutional Review Board of the Dana-Farber/Harvard Cancer Center. Using a random number generator, we selected 59 patients, categorized into 3 groups: control, comprising 20 patients without aGVHD after HSCT; skin, comprising 20 patients with primarily cutaneous aGVHD after HSCT (grade III or IV); and gut, comprising 19 patients with intestinal aGVHD after HSCT (grade III or IV). Patients in the gut group were allowed to have skin or liver aGVHD, whereas those in the skin group were not allowed to have greater than grade I disease of the intestine or liver. The diagnosis and grading of aGVHD were recorded previously and were based on the patients' clinical and pathological features, in accordance with previously published criteria [28]. The patients had been treated with either myeloablative (MA; either cyclophosphamide (Cy)/total body irradiation (TBI) or busulfan (Bu)/Cy) or reduced-intensity conditioning (RIC; Bu/Flu) regimens. Donor stem cells were obtained from either HLA-matched related donors (MRDs) or HLA-matched unrelated donors (MUDs). All patients received calcineurin inhibitor–based (either cyclosporine (CsA) or tacrolimus, dosed to target serum level) GVHD prophylaxis.

Blood Samples

All of the samples used in this study were obtained from a patient sample repository established at DFCI beginning in 2000 and were collected from patients who had signed informed consent to provide blood and bone marrow samples for research purposes. All samples were collected in EDTA tubes from patients after HSCT, and mononuclear cells were isolated by density gradient centrifugation. At the time of collection, cells were cryopreserved in FBS + 10% DMSO at −180°C. After appropriate patients were identified, samples were selected with the goal of using blood samples that had been collected within 30 days before the onset of aGVHD symptoms. After the blood samples were selected, peripheral blood mononuclear cells (PBMCs) were washed before analysis by flow cytometry.

Flow Cytometry

Cells were stained using a 7-color antibody panel comprising conjugated antibodies against CD45, CD3, CD4, CD8, CD45RO, α4β7 integrin, and CCR9. All of these reagents were purchased from Beckman Coulter (Fullerton, CA) except anti-CCR9, which was purchased from R&D Systems (Minneapolis, MN), and anti-α4β7 integrin (ACT-1), which was provided by Millennium Pharmaceuticals (Cambridge, MA) and conjugated at DFCI with phycoerythrin (PE). The cells were then analyzed using a Beckman Coulter FC500 or a FACSAria Special Order Research Platform (BD Biosciences, San Jose, CA) flow cytometer. T cell subsets were defined as follows: naïve $CD4^+$ T cells ($CD45^+$, $CD3^+$, $CD4^+$, and