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A paradoxical pattern of indoleamine 2,3-dioxygenase expression in the colon tissues of patients with acute graft-versus-host disease

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Indoleamine 2,3-dioxygenase (IDO) is a rate-limiting enzyme for tryptophan catabolism that plays an important role in the induction of immune tolerance. It is induced in the colon and exerts its effects there, regulating T-cell proliferation and survival. To address the role of IDO in acute graft-versus-host disease (AGVHD) after human allogeneic hematopoietic stem cell transplantation, we analyzed the relationship between IDO expression in colon tissues and clinical outcomes among 41 AGVHD patients who were diagnosed as gut AGVHD by a colon mucosal biopsy within 100 days posttransplantation. By in situ immunohistochemical analyses, IDO expression was measured in colon mucosal mononuclear cells (MNCs) and endothelial cells (ECs) in GVHD areas. High IDO expression in MNCs and low IDO expression in ECs had a trend toward a lower nonrelapse mortality ($p = 0.157$ and $p = 0.062$, respectively). Multivariate analysis showed that high MNC combined with low EC IDO expression ($p = 0.046$), as well as low disease risk ($p = 0.012$), are associated with lower nonrelapse mortality. Paradoxical upregulation of IDO expression in colon MNCs and ECs may represent a new predictive factor for prognosis in gut AGVHD after human allogeneic hematopoietic stem cell transplantation. © **2014 ISEH - International Society for Experimental Hematology. Published by Elsevier Inc.**

Allogeneic hematopoietic cell transplantation (allo-HSCT) is an important therapeutic option for a variety of malignant and nonmalignant disorders. However, acute graft-versus-host disease (AGVHD) remains a significant complication of allo-HSCT and limits the broader application of this therapy [1]. Indoleamine 2,3-dioxygenase (IDO) is an intracellular heme-containing enzyme that catalyzes the initial rate-limiting step in tryptophan degradation along the kynurenine pathway [2]. Decreased tryptophan and/or increased metabolite concentrations elicit a stress response in nearby responding T cells, leading to anergy or apoptosis [3]. The intestine, which is an important GVHD target organ, constitutively expresses IDO and can upregulate its expression during inflammation. Typically, antigen-presenting cells can be induced to express IDO during the intense inflammation of GVHD, and inhibition of functional IDO activity during immune-mediated colitis markedly worsens the disease [4]. Recently, IDO has been demonstrated to act as a critical regulator of GVHD in a mouse HSCT model, most strikingly in the colon, where colon epithelial cells were shown to dramatically upregulate IDO expression during GVHD [5]. In clinical allo-HSCT, increased IDO activity has been reported in the monocytes of allo-HSCT recipients without GVHD, and it has recently been reported in both duodenal epithelial cells and $CD4^+$ cells from patients with GVHD [6,7]. Moreover, strong activation of the IDO pathway was found by systematic analyses to be associated both with poor response to GVHD treatment and with severe GVHD [8]. Although induction of IDO was protective in experimental models of GVHD, the association of increased metabolite levels and IDO activity with GVHD severity and poor outcome observed in a human study seems contradictory. Here, we addressed the role of IDO in early complications after allo-HSCT by means of in situ immunohistochemistry in colon biopsies from patients with AGVHD. Our data suggest a

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strong association of paradoxical IDO expression in colon tissues with an outcome of clinical AGVHD.

Materials and methods

Patients and tissue samples

The objective of the present study was to investigate an association between IDO expression of colon mucosal biopsies and nonrelapse mortality (NRM) after allo-HSCT. In this retrospective study, we included 333 consecutive patients who had received an allo-HSCT at our institution between January, 2009, and June, 2010. Among these patients, clinically- and pathologically-confirmed AGVHD colon tissues from 41 patients could be consecutively collected during this period. Colon mucosal biopsy was performed at the time of first diagnosis within 100 days posttransplantation. All procedures were performed at the onset of gastrointestinal symptoms, and biopsies were done at the mucosal areas showing any abnormalities from erythematous to ulceration change. If multiple sites were biopsied, the most severely affected one was used for assessment. The clinical diagnosis of AGVHD and histologic grading were performed according to the method described in a previous report and according to a modification of the system for colonic GVHD described by Lerner (Fig. 1), respectively [9,10].

Immunohistochemical staining and analysis for indoleamine 2,3-dioxygenase expression

The expression of IDO in colon tissues was evaluated with immunohistochemistry. Briefly, IDO immunostaining was performed on 4-μm-thick paraffin sections of colonic mucosal biopsies. Paraffin sections were mounted on coated slides and deparaffinized, then rehydrated with downgraded ethanol. The slides were subjected to microwave with PTLink (Dako, Glostrup, Denmark) for antigen retrieval. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide for 5 min. The sections were incubated with mouse monoclonal anti-IDO Ab (clone 1F8.2, Millipore, Billerica, MA; dilution 1:500) for 30 min at room temperature. After washing, goat antimouse horseradish peroxidase (HRP) secondary Ab (MACH2 Mouse HRP-Polymer, Biocare, Concord, CA) was applied and further incubated for 20 min. The slides were visualized with Betazoid DAB Chromogen Kit (Biocare) and followed by hematoxylin counterstaining. To address the localization of IDO expression, double immunostaining was performed according to the manufacturer's protocol. Briefly, after antigen retrieval and endogenous peroxidase blocking, the sections were washed with TBST (Tris-buffered saline with 0.5% Tween-20; Sigma-Aldrich, St. Louis, MO) buffer. Both primary antibodies, mouse monoclonal anti-IDO Ab (dilution 1:200) and rabbit monoclonal anti-CD31 Ab (clone EP3095; Millipore, Billerica, MA; dilution 1:200) were applied simultaneously and incubated for 30 min at room

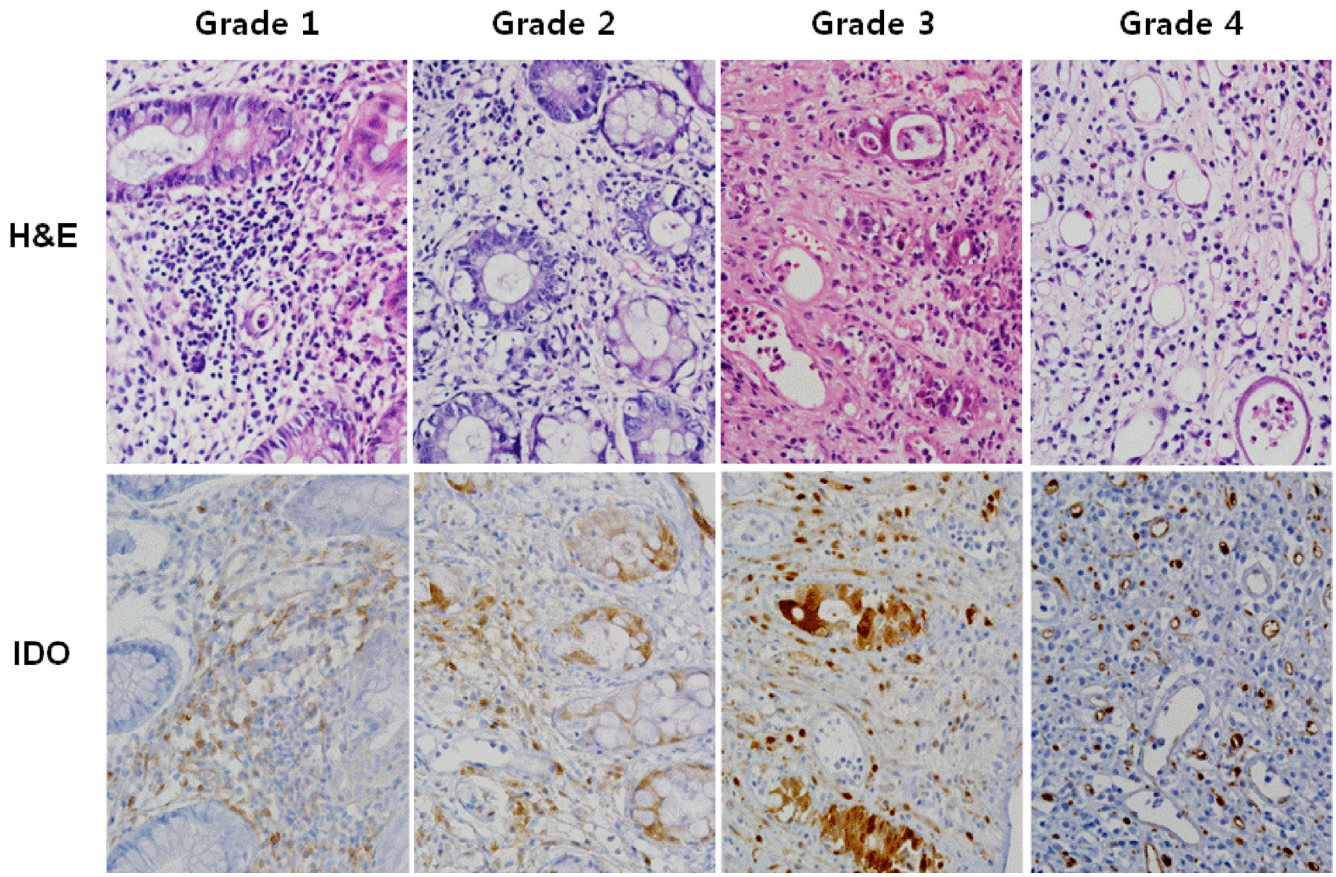

Figure 1. Histopathologic grading and representative IDO immunostaining for acute graft-versus-host disease: grade 1, individual epithelial apoptosis without complete crypt loss; grade 2, brisk apoptosis and sporadic crypt loss; grade 3, grouped loss of more than 2 crypts; and grade 4, diffuse crypt loss with mucosal erosion. Original magnification × 400. H&E = hematoxylin and eosin.