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Increased Plasma Indoleamine 2,3-Dioxygenase Activity and Interferon-γ Levels Correlate with the Severity of Acute Graft-versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Indoleamine 2,3-dioxygenase (IDO) is a rate-limiting enzyme for the tryptophan catabolism that plays an important role in the induction of immune tolerance. To evaluate the expression levels of IDO and interferon (IFN)-γ in patients receiving allogeneic hematopoietic stem cell transplantation (allo-HSCT) and to identify the correlation between IDO activity, IFN-γ, and acute graft-versus-host disease (aGVHD), we measured IDO mRNA expression in peripheral blood mononuclear cells in 89 allo-HSCT patients by reverse transcription-polymerase chain reaction. The IDO activity in plasma was also performed by reverse-phase high-performance liquid chromatography; plasma IFN-γ was detected by a standard enzyme-linked immunosorbent assay. IDO mRNA was detected in 55 of 74 patients (74.32%) with aGVHD. Of patients without aGVHD, only 2 of 26 expressed IDO mRNA (7.69%); none of 8 healthy volunteers was positive for IDO expression. Plasma IDO activity was much higher in aGVHD patients than in those without aGVHD (4.74 ± 3.35 vs 1.79 ± 1.02, respectively; $P < .0001$) or in healthy control subjects (4.74 ± 3.35 vs 1.77 ± .22; $P < .0001$). Patients with severe (grade III/IV) aGVHD had much higher IDO activity than those with mild (grade I/II) aGVHD (6.57 ± 3.34 vs 2.46 ± 1.41; $P < .0001$). Meanwhile, there was a significant increase in plasma IFN-γ level in aGVHD patients ($P = .0043$). IDO activity decreased after alleviation of aGVHD, whereas fluctuation of plasma IDO was also observed upon the recurrence of aGVHD. Plasma IDO activity was correlated with the level of plasma IFN-γ ($r = .8288$; $P < .0001$). Using receiver-operating characteristic curves analysis, the sensitivity and specificity for evaluation of aGVHD were determined. The area under the curve of IDO activity was higher than that of IFN-γ (.852 vs .694) with a sensitivity and specificity for IDO of 81% and 78%, respectively, whereas the sensitivity and specificity for IFN-γ were 41% and 93%, respectively. IDO mRNA was expressed in blood mononuclear cells of patients with aGVHD. Plasma IDO activity was elevated in aGVHD patients and was correlated with the severity of aGVHD. In combination with plasma IFN-γ, IDO activity may represent a potential biomarker for the diagnosis and evaluation of aGVHD after allo-HSCT. Intervention of the IDO pathway may also represent an alternative way to overcome steroid-resistant aGVHD.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most effective ways to cure hematopoietic malignancies, but acute graft-versus-host disease (aGVHD) remains the major challenge because it may incur HSCT-related complications and transplantation failure. Currently, no reliable instrumental or laboratory tests are available to evaluate the occurrence and the severity of aGVHD, so the implementation of optimal treatment strategies relies solely on clinical manifestation and subjective judgment. Finding objective parameters that may predict and distinguish aGVHD from other complications remains an important goal in the field of allo-HSCT. aGVHD is a typical donor type I helper T lymphocyte (T helper, type I, Th1)-mediated allogeneic immune response; Th1 cytokines, especially interferon (IFN)-γ produced by Th1 cells, are involved in several aspects of aGVHD pathophysiology [1].The role of IFN-γ in GVHD is dichotomous. On one hand, it plays key roles in the amplification of immune response and tissue damage in aGVHD [2]: In several studies, increased serum levels of IFN-γ were associated with the severity of aGVHD in mice [3-5]. On the other hand, IFN-γ mediates GVHD-associated immunosuppression in experimental GVHD. Brok et al. found that a high dosage of IFN-γ immediately after bone marrow transplantation was crucial for the prevention of aGVHD in a murine bone marrow transplantation model [6]. In view of its paradoxical roles in the pathogenesis of aGVHD and its spatiotemporal expression pattern, IFN-γ is not routinely measured in the circumstances of clinical aGVHD. Theoretically, it is possible that IFN-γ–related substances such as molecules in the downstream pathway of IFN-γ could be detected in case of aGVHD.

Indoleamine 2,3-dioxygenase (IDO) is an intracellular heme-containing enzyme that catalyzes the first and rate-limiting step of tryptophan catabolism along the kynurenine pathway. IDO is induced during inflammation by inflammatory cytokines; in particular, Th1 cytokine IFN-γ is the strongest inducer of IDO expression [7,8]. Tryptophan deficiency and/or metabolite concentration excesses have immunomodulatory effects, including suppression of

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response in nearby responding T cells through sensitization to apoptosis and inhibition of T cell activation [9]. Based on these properties, studies have shown that IDO plays a crucial role in the induction of immune tolerance during infection, maternal-fetal acceptance, tumor immunity, and transplantation [7,10,11]. In addition, IDO activity is upregulated in many Th1-mediated autoimmunity diseases, such as experimental autoimmune encephalomyelitis, autoimmune diabetes in nonobese diabetic mice, and collagen-induced arthritis [12-14]. Jasperson et al. showed that IDO−/− or IFN-γR−/− mice experienced more severe aGVHD after allogeneic bone marrow transplantation because these recipients were unable to produce IDO as an important counter-regulatory mechanism [15]. In allogeneic bone marrow transplantation recipients, increased IDO expression has been reported in monocytes, intestinal epithelial cells, and plasma from patients with aGVHD [9,16,17]. Landfried et al. found that tryptophan metabolite levels were elevated in the urine of patients with GVHD, and increased expression of IDO mRNA was detected in intestinal biopsies from patients with severe GVHD. Levels of kynurenine and IDO mRNA were correlated with GVHD severity [18]. Although these studies indicate that IDO expression is upregulated in the target organ and plasma of aGVHD patients, systematic analyses and dynamic monitoring methods are still lacking. Therefore, we collected peripheral blood samples from patients before and after HSCT to measure the concentrations of kynurenine and tryptophan in plasma and to detect the IDO mRNA expression in peripheral blood mononuclear cells. Our results indicate that the plasma IDO activity is correlated with the severity and progression of aGVHD.

Table 1
Patient Characteristics

Characteristics	No. of Patients with Characteristic for Indicated Grade of aGVHD (%)		
	Grade 0-II	Grade III-IV	*P* Value
Total patient No.	58	31	—
Sex			
Male	35 (60)	12 (39)	.195
Female	23 (40)	19 (61)	
Age, y			
Median	33	28	.853
Range	3-55	12-42	
Disease			
AML	21 (36)	10 (32)	
ALL	11 (19)	7 (23)	
CML	9 (16)	4 (13)	.451
MDS	6 (10)	4 (13)	
SAA	7 (12)	2 (6)	
NHL	3 (5)	2 (6)	
Other diseases	1 (2)	2 (6)	
Conditioning regimen			
Myeloablative	45 (78)	21 (68)	.550
Reduced intensity	13 (22)	10 (32)	
Donor			
Match related	40 (69)	22 (71)	
Mismatched related	8 (14)	4 (13)	.917
Match unrelated	10 (17)	5 (16)	
GVHD prophylaxis			
MTX+CSA	37 (64)	16 (52)	
MTX+FK506	18 (31)	13 (42)	.264
MMF+CSA	3 (5)	2 (6)	

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anemia; NHL, non-Hodgkin lymphoma; aGVHD, acute graft-versus-host disease; MTX, methotrexate; CSA, cyclosporine A; FK506, tacrolimus; MMF, mycophenolate mofetil.

MATERIALS AND METHODS

Patients

A total of 89 patients undergoing allo-HSCT from June 2009 to January 2012 were enrolled in this study at the Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. This study was approved by the Medical Ethics Committee of the Tongji Hospital. All patients gave written informed consent to sampling of blood and collection of clinical data in accordance with the Declaration of Helsinki. The characteristics of all selected patients are summarized in Table 1.

Specimens

Blood samples from 89 patients receiving allo-HSCT and 8 healthy volunteers were collected, separated, and stored for further study. All samples were collected in EDTA tubes from patients on the day before HSCT and on days 30 and 60 post-HSCT. For patients with aGVHD, blood samples were also collected at the onset of aGVHD and after treatment. Samples from patients with infection were excluded from this study. The diagnoses and aGVHD grades were previously recorded based on patients' clinical and pathological features and laboratory data, in accordance with previously published criteria [19]. Peripheral blood was centrifuged at 1,500 rpm for 5 minutes to separate the plasma, which was then divided into 2 parts. One part was stored at −80°C in preparation for the measurement of IFN-γ, and the other part was deproteinized with an equal volume of .5N perchloric acid for 10 minutes on ice and centrifuged at 15,000 rpm for 10 minutes to recover the supernatant for the measurement of kynurenine and tryptophan.

Measurement of Kynurenine and Tryptophan Levels

Tryptophan and kynurenine concentrations (μmol/L) in plasma were measured by reverse-phase high-performance liquid chromatography (Waters, Milford, MA) as described previously [20]. In brief, the frozen supernatants were thawed at room temperature and separated on a C18 reversed-phase column; the mobile phase was 15 mmol/L acetic acid/sodium acetate (pH 4.0) containing 27 mL/L acetonitrile. Tryptophan was detected by its native fluorescence at 287-nm excitation and 357-nm emission. Kynurenine was monitored by ultraviolet absorption at 360-nm wavelength in the same chromatographic run. The plasma kynurenine concentrations and tryptophan concentrations were twice the values indicated by the high-performance liquid chromatography equipment. The IDO activity was calculated by dividing the plasma concentration of kynurenine by that of tryptophan, and the ratio was normalized (×100).

Determination of Plasma IFN-γ Levels

To determine the IFN-γ levels and any association between IFN-γ and IDO activity in patients undergoing transplantation, especially in the cases of aGVHD, plasma IFN-γ was measured using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). Assays were performed according to the manufacturer's protocol and read at 450 nm by a microplate reader (Bio-Rad, Berkeley, CA).

Reverse Transcription–Polymerase Chain Reaction Analysis of IDO in Mononuclear Cells

Mononuclear cells were separated by density- gradient centrifugation; total RNA was then isolated using a Trizol reagent (Invitrogen, Carlsbad, CA) and was then reverse transcribed into cDNA using a transcription kit (Fermentas, Thermo-Fisher Scientific, Barrington, IL) in a total volume of 25 μL at 42°C for 60 minutes, extended at 95°C for 5 minutes. The cDNA was amplified by polymerase chain reaction (PCR) using IDO sense 5′-TTTCAC-CAAATCCACGAT CA-3′ and antisense 5′-CAGGACGTCAAAGCACTGAA-3′ primers. After an initial denaturation step of 5 minutes at 95°C, 35 cycles consisting of 30 seconds at 94°C, 30 seconds at 59°C, and 45 seconds at 72°C were performed, followed by a final extension of 10 minutes at 72°C. PCR products were resolved on 2% agarose gel stained with ethidium bromide and visualized on a Chemi-Imager 4400 system (Alpha Innotech Co., Johannesburg, South Africa).

Statistical Analyses

Data are presented as mean ± standard deviation. IDO activity and IFN-γ levels were analyzed using Kruskal-Wallis, analysis of variance, and Mann-Whitney U test. Probability values of $P < .05$ were interpreted as statistically significant. Correlation between IDO activity and IFN-γ level was assessed by linear regression. Multivariable logistic regression was analyzed for patient characteristics; the receiver-operating characteristic (ROC) analysis was used to evaluate the sensitivity and specificity of IDO activity and IFN-γ