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Donor Nucleotide-Binding Oligomerization–Containing Protein 2 (NOD2) Single Nucleotide Polymorphism 13 Is Associated with Septic Shock after Allogeneic Stem Cell Transplantation

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Single nucleotide polymorphisms (SNPs) within nucleotide-binding oligomerization domain–containing protein 2 (NOD2) and toll-like receptor (TLR) 5 genes have been recently associated with the incidence and outcome of infections. In this study, we analyzed 38 patients with septic shock after allogeneic stem cell transplantation (allo-SCT) for an association of SNPs within NOD2 and TLR5 genes, with susceptibility to septic shock. One hundred twenty-seven transplant recipients unaffected by any infectious complications were used as controls. We found a significant association between the presence of donor NOD2 SNP13 (3016_3017insC) and the incidence of septic shock ($P = .002$). In multivariate analysis, donor NOD2 SNP13 appeared as an independent risk factor for the incidence of septic shock after allo-SCT. No association was found for recipient SNPs (NOD2 and TLR5) and donor NOD2 SNP8, SNP12, and TLR5-Stop SNP. Our results suggest that NOD2 SNP13 has an impact on the pathophysiology of severe infectious complications and is an independent risk factor for the development of septic shock after allo-SCT.

INTRODUCTION

Despite an improvement in therapy in the last years, sepsis syndrome remains a severe complication and is 1 of the leading causes of death in patients after allogeneic stem cell transplantation (allo-SCT) [1-5]. After allo-SCT, patients are at increased risk because of various factors, such as prolonged neutropenia, impairment of the mucosal barrier, acute or chronic graft-versus-host disease (GVHD), and distinct immunosuppression with delayed recovery of T and B cell function [6,7].

However, not all individuals sharing the same risk factors develop sepsis syndrome and it is suggested that genetic variations might contribute to the susceptibility of infection. Recently, it has been described that single nucleotide polymorphisms (SNPs) within genes of pattern recognition receptors (PRR), such as toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD)-like receptors, which are expressed by cells of the innate immune system to recognize microbial antigens, are associated with the incidence and outcome of infections [8-16].

The NOD-containing protein 2/caspase recruitment domain-containing protein 15 (NOD2) is an intracellular sensor of muramyl dipeptide, a cell wall component of both gram-positive and gram-negative bacteria [17]. Furthermore, NOD2 has recently been identified as cytoplasmatic PRR for viral antigens [18]. NOD2 is expressed in various cell types involved in the microbial defense [19-25]. By activating nuclear factor kappa B (NF-κB), NOD2 mediates the induction of antimicrobial peptides (defensins) and induces an inflammatory response in epithelial cells [26]. Our group has previously shown that SNPs within the NOD2 gene are independent risk factors for transplantation-related mortality, development of GVHD and bronchiolitis obliterans after allo-SCT, and early mortality of nontransplantation patients with

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sepsis syndrome [8,27,28]. Furthermore, others have demonstrated that SNPs within the NOD2 gene are associated with the susceptibility to various bacterial infections [15,16,29] and sepsis syndrome [8,12,15,30].

TLR5 is expressed by several cell types involved in the microbial immune defense (eg, bronchial epithelial cells, monocytes, neutrophils, dendritic cells, and T cells) and is able to recognize a wide variety of pathogenic bacteria by binding to a highly conserved region of bacterial flagellin [31]. Activation of TLR5 induces an inflammatory response of different cells of the immune system. An SNP within the ligand binding domain of the TLR5 gene that results in a premature stop codon (common stop codon polymorphism, TLR5-Stop SNP) and abrogates TLR5 signaling is associated with susceptibility to various bacterial infections [31-33]. Recently, our group demonstrated a significant association with recipient TLR5-Stop SNP and the incidence of invasive aspergillosis after allo-SCT [34].

Because both NOD2 and TLR5 are involved in the immune response to infections, we investigated whether donor and recipient SNPs within the NOD2 and TLR5 gene are associated with the incidence of septic shock in patients after allo-SCT.

MATERIAL AND METHODS

Patients

A total of 165 patients and their donors were analyzed in this retrospective study. The individuals underwent allo-SCT between 1998 and 2011 at the University Hospital of Regensburg and were enrolled in a clinical investigation approved by the institutional research ethics committee. Blood samples to perform allelic discrimination tests were collected after informed consent before transplantation. Conditioning and prophylactic immunosuppression was performed according to standard protocols. Standard conditioning regimens consisted of 8 to 12 gray fractionated total body irradiation followed by high-dose cyclophosphamide or classic busulfan/cyclophosphamide. Reduced-intensity conditioning consisted mainly of fludarabine/BCNU and melphalan regimens. Patients receiving allo-SCT from unrelated donors underwent in vivo T cell depletion with antithymocyte globulin in the context of pretransplantation conditioning. All patients have been treated with gastrointestinal decontamination (GD) addressing mainly gram-negative and anaerobic bacteria (ciprofloxacin and metronidazole starting from day +1).

Microbiological cultures (eg, from whole blood, urine, or bronchoalveolar lavage) were performed according to standard techniques. Blood cultures were considered positive if a micro-organism was detected in at least 1 bottle, except for coagulase-negative staphylococci, for which 2 separate positive blood cultures with the same strain were required.

Among the analyzed individuals were 38 patients with septic shock after allo-SCT according to international criteria (≥2 Systemic Inflammatory Response Syndrome [SIRS] criteria, known or suspected infection, and hypotension unresponsive to fluid resuscitation alone) [35-37]. Gram-positive bacteria were identified in 19 individuals (50%) and gram-negative were identified in 17 (45%). In 2 patients (5%), cytomegalovirus was identified as causing pathogen (atypical pneumonia). Analyzing clinical symptoms and imaging test results (eg, computed tomography scan or ultrasound) in patients with septic shock, we found the gastrointestinal tract (17 patients, 45%), the lung (15 patients, 39%), and the urinary tract (2 patients, 5%) to be the primary sources of infection. In 4 individuals (11%), no focus of infection could be identified. However, a pathogen could be identified in all of these individuals. Patients with septic shock before allo-SCT were excluded from the study. We used 127 allo-SCT patients unaffected by septic shock and any infectious complications as controls.

Individuals were of Caucasian ethnic background only (99 males and 66 females; median age, 47 years) and patients suffered from acute myelogenous leukemia (n = 63), non-Hodgkin lymphoma (n = 24), chronic myelogenous leukemia (n = 12), acute lymphoblastic leukemia (n = 19), multiple myeloma (n = 19), osteomyelofibrosis (n = 9), chronic lymphocytic leukemia (n = 7), myelodysplastic syndrome (n = 7), Hodgkin disease (n = 2), or other hematological/oncological diseases (breast cancer [n = 1] and severe aplastic anemia [n = 2]). There was no significant difference between patients with and without septic shock (controls) in terms of clinical features except grade of GVHD. Clinical characteristics are shown in Table 1.

Table 1
Patients and Clinical Characteristics

Characteristic	Control	Septic Shock	*P* Value
Disease			
AML	45 (35)	18 (47)	
NHL	19 (15)	5 (13)	
CML	10 (8)	2 (5)	
ALL	13 (10)	6 (16)	
MM	16 (13)	3 (8)	
OMF	8 (6)	1 (3)	
CLL	7 (5)	0	
MDS	5 (4)	2 (5)	
Hodgkin disease	2 (2)	0	
Others*	2 (2)	1 (3)	
Gender			.11
Male	72 (57)	27 (71)	
Female	55 (43)	11 (29)	
Age, mean (range)	47 (18-69)	49 (18-67)	
Stem cell source			.21
Bone marrow	14 (11)	5 (13)	
Peripheral blood	113 (89)	33 (87)	
Conditioning			.80
Standard	34 (27)	11 (29)	
Reduced intensity	93 (73)	27 (71)	
Donor type			.70
Unrelated	65 (51)	18 (47)	
Sibling	62 (49)	20 (53)	
Disease stage			.30
Early/intermediate	75 (59)	19 (50)	
Advanced	52 (41)	19 (50)	
GVHD			**<.001**
Grade 0-II	123 (97)	21 (55)	
Grade III-IV	4 (3)	17 (45)	

AML indicates acute myelogenous leukemia; NHL, non-Hodgkin lymphoma; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; MM, multiple myeloma; OMF, osteomyelofibrosis; CLL, chronic lymphocytic leukemia; MDS, myelodysplastic syndrome.
P value calculated by chi-square test. Control group did not have septic shock. Significant *P* values are shown in bold.
* Others: 2 severe aplastic anemia and 1 breast cancer.

Collection of DNA Samples and Typing for SNPs

NOD2 2104C>T (SNP8; Arg702Trp; rs2066844), 2722G>C (SNP12 Gly908Arg; rs2066845), 3016_3017insC (SNP13; rs2066847) [28], and TLR5 1174C>T (TLR5-Stop SNP; Arg392Ter; rs5744168) [31,38] gene polymorphisms were determined by Taqman allelic discrimination test as previously described [28,38,39]. DNA from individuals has been isolated from EDTA blood. Samples were analyzed for association between risk for septic shock and the particular PRR gene polymorphisms.

Statistical Analysis

Hardy-Weinberg equilibrium analysis was performed by chi-square test for each SNP by comparing the detected genotype distribution with the theoretical distribution estimated on the basis of the SNP allelic frequencies. Donor and recipient allele and genotype frequencies between patients with septic shock and controls were compared by chi-square test and the cumulative incidence of septic shock was calculated by the Kaplan-Meier method. In cases where the expected values in any of the cells of the contingency table were below 5, the Fisher exact test was used instead of chi-square test.

Multivariate analysis was performed by Cox regression calculation. *P* values less than .05 were considered statistical significant. Values of hazard ratio, *P*, and chi-square were calculated at 95% confidence intervals (CI).

RESULTS

Frequency of NOD2 SNPs and TLR5-STOP SNP

We first analyzed the frequency of NOD2 SNP8, SNP12, SNP13, and the TLR5-Stop SNP in the 165 recipients and their donors by allelic discrimination. In all of the analyzed individuals, genotype frequencies did not deviate from those predicted by the Hardy-Weinberg equilibrium ($P > .05$). Only heterozygous and no homozygous mutations were observed in the study population.

NOD2 allele frequencies for SNP8 were 4.5% for recipients and 5.2% for donors, those for SNP12 were 1.8% for recipients and donors, and those for SNP13 were 2.4% for recipients and