MAJOR ARTICLE

Cytokine Gene Polymorphisms and the Outcome of Invasive Candidiasis: A Prospective Cohort Study

Melissa D. Johnson,[1,2,a] Theo S. Plantinga,[3,4,a] Esther van de Vosse,[6] Digna R. Velez Edwards,[7] P. Brian Smith,[1] Barbara D. Alexander,[1] John C. Yang,[8] Dennis Kremer,[9] Gregory M. Laird,[1] Marije Oosting,[3,4] Leo A. B. Joosten,[3,4] Jos W. M. van der Meer,[3,4] Jaap T. van Dissel,[6] Thomas J. Walsh,[10] John R. Perfect,[1] Bart-Jan Kullberg,[3,4] William K. Scott,[5] and Mihai G. Netea[3,4]

[1]Duke University Medical Center, Durham, [2]Department of Clinical Research, Campbell University College of Pharmacy and Health Sciences, Buies Creek, North Carolina; [3]Department of Medicine, and [4]Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Center, the Netherlands; [5]John P. Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Florida; [6]Department of Infectious Diseases, Leiden University Medical Center, the Netherlands; [7]Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, Tennessee; [8]National Jewish Health, Denver, Colorado; [9]Department of Molecular Epidemiology, Leiden University Medical Center, the Netherlands; and [10]Cornell University, New York

Background. *Candida* bloodstream infections cause significant morbidity and mortality among hospitalized patients. Although clinical and microbiological factors affecting prognosis have been identified, the impact of genetic variation in the innate immune responses mediated by cytokines on outcomes of infection remains to be studied.

Methods. A cohort of 338 candidemia patients and 351 noninfected controls were genotyped for single-nucleotide polymorphisms (SNPs) in 6 cytokine genes (*IFNG*, *IL10*, *IL12B*, *IL18*, *IL1β*, *IL8*) and 1 cytokine receptor gene (*IL12RB1*). The association of SNPs with both candidemia susceptibility and outcome were assessed. Concentrations of pro- and antiinflammatory cytokines were measured in in vitro peripheral blood mononuclear cell stimulation assays and in serum from infected patients.

Results. None of the cytokine SNPs studied were associated with susceptibility to candidemia. Persistent fungemia occurred in 13% of cases. In the multivariable model, persistent candidemia was significantly associated with (odds ratio [95% confidence interval]): total parenteral nutrition (2.79 [1.26–6.17]), dialysis dependence (3.76 [1.46–8.64]), and the SNPs *IL10* rs1800896 (3.45 [1.33–8.93]) and *IL12B* rs41292470 (5.36 [1.51–19.0]). In vitro production capacity of interleukin-10 and interferon-γ was influenced by these polymorphisms, and significantly lower proinflammatory cytokine concentrations were measured in serum from patients with persistent fungemia.

Conclusions. Polymorphisms in *IL10* and *IL12B* that result in low production of proinflammatory cytokines are associated with persistent fungemia in candidemia patients. This provides insights for future targeted management strategies for patients with *Candida* bloodstream infections.

Invasive candidiasis is a pervasive nosocomial infection. Although some patients may experience only transient fungemia, others develop complications including endocarditis, abscesses, and chronic-disseminated candidiasis. Persistent fungemia is an increasingly recognized complication of candidemia, which occurs in 8%–15% of candidemia patients. Few studies have provided an explanation for these differential outcomes, although immune response to infection, comorbidities, and pharmacologic therapy have all been suggested to contribute to patient outcomes [1].

Both innate and adaptive immune mechanisms are important for host defense against *Candida* species [2]. The innate immune system provides the first line of defense against fungal pathogens by phagocytosis

Received 13 April 2011; accepted 4 October 2011; electronically published 5 December 2011.

Presented in part: Oral presentation at ECCMID, Vienna, Austria, 12 April 2010.

[a]M. D. J. and T. S. P. contributed equally to the study.

Correspondence: Theo.S. Plantinga, MSc, Department of Medicine, Radboud University Nijmegen Medical Centre, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, the Netherlands (t.plantinga@aig.umcn.nl).

Clinical Infectious Diseases 2012;54(4):502–10

DOI: 10.1093/cid/cir827

and killing of invading pathogens, as well as through activation of adaptive immunity through antigen presentation and secretion of proinflammatory cytokines [3]. Adaptive fungal immunity stimulates host responses to pathogens via protective cellular T-helper 1 (Th1) cytokines, such as interferon-γ (IFN-γ), and humoral Th2 responses that may have maladaptive antiinflammatory effects by the release of interleukin (IL)–4 and IL-10. The interplay between Th1 and Th2 responses to *Candida* infection is complex but critical for the response to this pathogen. Previous studies have demonstrated that vigorous Th1-type responses are essential for eradication of *Candida* [4, 5]. In contrast, Th2-type responses to *Candida* may lead to down-regulation of proinflammatory cytokines, thereby terminating the protective response to infection [6].

Genetic factors are known to have an important impact on susceptibility to infections [7]. Although much has been learned about the immune mechanisms that determine an effective host defense against *Candida*, very little is known about the role played by single-nucleotide polymorphisms (SNPs) of genes comprising the cytokine network for susceptibility to systemic candidiasis. This study was undertaken to investigate the role of polymorphisms in the main cytokine genes for the susceptibility to *Candida* infection and to assess whether there is any association with the subsequent clinical outcome.

METHODS

Study Design

This was a prospective, observational cohort study conducted at Duke University Medical Center (DUMC) and Radboud University Nijmegen Medical Center (RUNMC). Subjects were enrolled after informed consent (or waiver as approved by the institutional review boards) at DUMC (Durham, North Carolina) and RUNMC (Nijmegen, the Netherlands). The study was approved by the institutional review board at each study center, and enrollment took place between January 2003 and January 2009. The study was performed in accordance with the Declaration of Helsinki.

Subjects

Infected adults (≥18 years of age) were identified by report of ≥1 positive blood cultures for *Candida* species in the clinical microbiology laboratory at the participating center. Noninfected controls recruited at DUMC must have been hospitalized with no history or evidence of candidemia/invasive candidiasis or any other invasive fungal infection. Noninfected controls at DUMC were recruited from the same hospital wards as infected patients so that comorbidities and clinical risk factors for infection would be as similar as possible between groups. Noninfected controls enrolled at RUNMC were nonhospitalized healthy controls. Subjects were excluded from the study if insufficient volume of blood or clinical data were available. Intergroup comparisons between the 2 groups of noninfected subjects and between the 2 groups of infected subjects (at DUMC and RUNMC) were performed regarding similarity in genetic distribution of the studied SNPs prior to further statistical analysis of infected versus noninfected subjects.

Procedures

Plasma, serum, and whole blood specimens were obtained in combination with routine blood draws at baseline and during a 12-week follow-up period. Blood cultures were drawn as part of routine care until clearance of fungemia was documented or death occurred. For genetic analysis, DNA was isolated from whole blood by standard procedures. For in vitro peripheral blood mononuclear cell (PBMC) stimulation assays, blood was drawn from healthy volunteers recruited at RUNMC.

Outcomes

Presence of invasive candidiasis was determined on the basis of the European Organization for Research and Treatment of Cancer definitions. All-cause mortality was determined based on hospital records and, if necessary for DUMC subjects, the Social Security Death Index. Disseminated disease was defined as the presence of *Candida* species at normally sterile sites outside the bloodstream (excluding the urine) and further characterized as acute or chronic. Persistent fungemia was defined as ≥5 days of persistently positive blood cultures for the same *Candida* species. All infected patients were followed for up to 12 weeks to assess for clinical outcome.

Predictor Variables

Within the DUMC infected cohort, numerous baseline clinical and microbiological variables were assessed for their relationship to study outcomes. These included age, race, sex, and immunocompromised host status (see Table 2).

Genotyping

Mostly SNPs in promoter and 5′-untranslated regions of the analyzed genes were selected on the basis of previously established associations with human diseases or known functional effects on protein function or gene expression. SNPs in 6 cytokine genes (*IL1β*, *IL8*, *IL10*, *IL12B*, *IL18*, *IFNG*) and 1 cytokine receptor gene (*IL12RB1*) were genotyped (Supplementary Table 1) by using a mass-spectrometry genotyping platform (Sequenom, LUMC). Quality control was performed by duplicating samples within and across plates and by incorporation of positive control samples.

In Vitro PBMC Stimulation Assays

Isolation and stimulation of PBMCs was performed as described elsewhere [8]. In brief, PBMCs obtained from individuals were incubated at 37°C for 48 hours with either culture medium or with *Candida albicans* conidia or hyphae (strain

UC820, both 10^6/mL). Production of IL-10 and IFN-γ was measured in PBMC supernatants by enzyme-linked immunosorbent assay (R&D Systems and Sanquin).

Cytokine Assays

Cytokine concentrations of IL-6, IL-8, IFN-γ, IL-10, and IL-12p40 in plasma and serum samples obtained from infected patients from day 0 to day 7 after initial positive blood culture were measured by Multiplex Fluorescent Bead Immunoassays (xMAP technology, Bio-Rad) and a Bio-plex microbead analyzer (Luminex) according to the manufacturer's protocol. Day 0 was defined as the date that the first positive blood culture was drawn.

Statistical Analysis

For the genetic analysis, statistical tests for single locus association and for deviations from Hardy-Weinberg equilibrium (HWE) for all SNPs were calculated using PLINK statistical software [9]. Statistical significance for deviations from HWE in cases and controls were determined using χ^2 tests. Single locus tests of association were performed with logistic regression using both a dominant and a recessive model. Standard summary statistics (odds ratio [OR] and 95% confidence interval [CI]) were reported for these tests of association. Pairwise linkage disequilibrium (LD), D′ and r^2, were calculated using Haploview software [10]. Haplotype blocks were assigned using the D′ confidence interval algorithm created by Gabriel et al [11]. Data on susceptibility to infection were analyzed for white descendents separately from blacks because allelic frequencies were expected to differ among these 2 populations and controls.

Within the infected DUMC cohort, allelic frequencies were further assessed in association with 3 prespecified clinical outcomes: (1) disseminated disease, (2) persistent fungemia, and (3) all-cause mortality at 30 days. This analysis was performed in the entire cohort, and race was considered as a covariate in the analysis. Variables with P values $<.2$ in the univariate analysis or thought to be clinically important were further assessed in multivariable logistic regression models. Only variables with P values $<.05$ were retained in the final predictive model. ORs and 95% CIs were reported for variables that remained significant in the final multivariable model.

For the analysis of cytokine concentrations in the in vitro PBMC stimulation assays and in serum/plasma from the infected patients, Mann-Whitney U tests were performed to test for statistical differences. All tests were 2-sided.

RESULTS

Baseline Characteristics

In total, 338 infected (298 DUMC, 40 RUNMC) and 351 (300 DUMC, 51 RUNMC) noninfected adults were included (Table 1). The white patient group consisted of 237 infected and 263 noninfected subjects.

Table 1. Demographics for Duke University Medical Center and Radboud University Nijmegen Medical Center Adult Study Subjects

Demographic	Infected Subjects (n = 338), % (No.)	Noninfected Controls (n = 351), % (No.)
Race		
White	70 (237)	75 (263)
Black	28 (93)	25 (88)
Other	2 (8)	0 (0)
Sex		
Male	59 (199)	52 (184)
Female	41 (139)	48 (167)

Clinical characteristics of infected and noninfected subjects from DUMC were similar (Table 2). The following *Candida* species were commonly identified in the blood of infected subjects: *C. albicans* (42%), *C. glabrata* (29%), *C. parapsilosis* (16%), *C. tropicalis* (13%), *C. krusei* (4%). In sum, 5% of subjects had >1 *Candida* species isolated. Persistent fungemia was found in 13% of individual cases. Disseminated disease was observed in 18% of infected cases, and 30-day mortality was 29%.

Susceptibility to Infection

All infected patients and noninfected controls were successfully genotyped for all variants. All variants were in HWE (data not shown). The intergroup comparison between the Dutch RUNMC and Caucasian DUMC noninfected controls and between the infected subjects recruited at DUMC and RUNMC revealed a similar genetic distribution of the genotyped SNPs, which allowed the groups to be merged into 1 group of noninfected controls and 1 group of infected subjects (data not shown). Allelic frequencies of the cytokine SNPs did not differ significantly between infected and noninfected white subjects (Table 3). Similar results were obtained in the European-American group, when analyzed separately (not shown).

Similarly, no association between cytokine polymorphisms and susceptibility to bloodstream *Candida* infections was observed in the black cohort (Table 4). Therefore, none of the cytokine SNPs studied were associated with susceptibility to candidemia.

Univariate Analysis of Clinical Outcomes

In the DUMC infected cohort, variables that were significantly associated with persistent fungemia on univariate analysis included immunocompromised status, receipt of TPN, and *C. parapsilosis* infection. Of the cytokine SNPs tested, polymorphisms in *IL10* and *IL12B* genes were associated with persistent fungemia using univariate analysis in a recessive model (Table 5). In contrast, no association was observed

Table 2. Baseline Patient Characteristics for Duke University Medical Center Infected Subjects and Controls, including White and Black Adults

Variable	Infected Subjects (n = 298), %	Noninfected Controls (n = 300), %	*P* Value
Mean age, years (SD)	55.9 (16.8)	57.8 (16.4)	.31
Immunocompromised state	59	38	<.0001
HSCT	1	0	.12
Solid organ transplant	12	2	<.0001
Active malignancy[a]	32	20	.002
Solid tumor	23	12	
Leukemia	7	5	
Lymphoma	4	4	
Chemotherapy within past 3 months	17	11	.03
Neutropenia (ANC <500 cells/mm^3)	10	4	.001
HIV-infected	1	0	.06
Surgery within past 30 days	43	48	.25
Receipt of total parenteral nutrition	19	4	<.0001
Dialysis dependent	12	7	.02
Acute renal failure	34	22	.001
Liver failure	25	4	<.0001
ICU admission within past 14 days	49	34	<.0001
Median baseline serum creatinine (mg/dL)	1.3	1.0	.001
Median baseline WBC count (cells/mm^3)	10.55	8.55	.04
Candida species[b]			
albicans	42	…	
glabrata	29		
parapsilosis	16		
tropicalis	13		
krusei	4		
Other *Candida* species	3		
>1 *Candida* species	5		

Abbreviations: ANC, absolute neutrophil count; HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit; SD, standard deviation; WBC, white blood cell.

[a] Subjects could have more than one.

[b] Sixteen subjects had >1 species isolated.

Table 3. Associations of Polymorphisms in *IFNG, IL10, IL12B, IL18, IL1B, IL8,* and *IL12RB1* and Susceptibility to Candidemia in White Adults[a]

Polymorphism	Wild Type	Heterozygous	Homozygous Mutant	*P* Value
IFNG rs2430561	TT	TA	AA	.07
Noninfected, %	30.8	46	23.2	
Infected, %	23.4	56.6	20	
IL10 rs1800872	GG	TG	TT	.50
Noninfected, %	61.1	32.8	6.1	
Infected, %	58.3	35.3	6.4	
IL10 rs1800896	AA	GA	GG	.98
Noninfected, %	24.4	47.7	27.9	
Infected, %	27.2	45.1	27.6	
IL12B rs41292470	INS INS	INS DEL	DEL DEL	.11
Noninfected, %	23.6	47.7	28.7	
Infected, %	24.8	52.9	22.2	
IL12B rs3212227	AA	CA	CC	.95
Noninfected, %	64.8	32.9	2.3	
Infected, %	64.7	31.0	4.3	
IL12RB1 rs17882232	CC	TC	TT	.34
Noninfected, %	67.2	28.2	4.6	
Infected, %	62.8	33.8	3.4	
IL12RB1 rs17884858	AA	GA	GG	.67
Noninfected, %	48.1	41.8	10.2	
Infected, %	45.9	43.3	10.8	
IL18 rs187238	GG	GC	CC	.85
Noninfected, %	53.6	35.9	10.5	
Infected, %	51.9	41.3	7.9	
IL18 rs549908	TT	TG	GG	.78
Noninfected, %	48.1	40.1	11.8	
Infected, %	49.2	41	9.8	
IL1B rs1143634	GG	GA	AA	.85
Noninfected, %	59.9	33.6	6.5	
Infected, %	60.5	36.1	3.4	
IL1B rs16944	AA	GA	GG	.42
Noninfected, %	15.95	45.5	38.5	
Infected, %	11.5	46.6	41.9	
IL8 rs4073	AA	TA	TT	.86
Noninfected	18	54	27.9	
Infected	19.9	51.1	29	

[a] Includes 245 infected and 263 noninfected subjects.

between polymorphisms in cytokine genes and disseminated disease or 30-day mortality (data not shown).

Multivariate Logistic Regression Analysis of Persistent Fungemia

In the multivariable model, the following variables were analyzed within the logistic regression model and were significantly associated with persistent candidemia (OR [95% CI]): TPN (2.79 [1.26–6.17]), dialysis dependence (3.76 [1.46–8.64]), and the following SNPs: *IL10* rs1800896 (GG or GA vs AA, 3.45 [1.33–8.93]) and *IL12B* rs41292470 (INS/DEL or DEL/DEL vs INS/INS, 5.36 [1.51–19.0]).

In Vitro Cytokine Production by PBMCs

Stimulation of PBMCs obtained from healthy individuals bearing different *IL10* rs1800896 genotypes with either *C. albicans* conidia or *C. albicans* hyphae for 48 hours revealed increased production

Table 4. Associations of Polymorphisms in *IFNG, IL10, IL12B, IL18, IL1B, IL8,* and *IL12RB1* and Susceptibility to Candidemia in Black Adults[a]

Polymorphism	Wild Type	Heterozygous	Homozygous Mutant	*P* Value
IFNG rs2430561	TT	TA	AA	.12
Noninfected, %	69.3	25.0	5.7	
Infected, %	56.9	40.9	2.15	
IL10 rs1800872	GG	TG	TT	.23
Noninfected, %	41.4	37.9	20.7	
Infected, %	33.3	47.3	19.4	
IL10 rs1800896	AA	GA	GG	.52
Noninfected, %	47.1	42.5	10.3	
Infected, %	41.9	47.3	10.8	
IL12B rs41292470	INS INS	INS DEL	DEL DEL	.45
Noninfected, %	17.1	48.9	34.1	
Infected, %	12.9	45.2	41.9	
IL12B rs3212227	AA	CA	CC	.11
Noninfected, %	37.5	47.7	14.8	
Infected, %	50.5	37.6	11.8	
IL12RB1 rs17882232	CC	TC	TT	.67
Noninfected,%	68.9	27.6	3.5	
Infected, %	66.7	29.0	4.3	
IL12RB1 rs17884858	AA	GA	GG	.74
Noninfected, %	77.7	22.3	0	
Infected, %	75.8	20.9	3.3	
IL18 rs187238	GG	GC	CC	.11
Noninfected, %	69.4	27.1	3.5	
Infected, %	59.3	30.9	9.9	
IL18 rs549908	TT	TG	GG	.39
Noninfected, %	62.8	34.9	2.3	
Infected, %	66.9	37.6	6.5	
IL1B rs1143634	GG	GA	AA	.23
Noninfected, %	73.6	22.9	3.5	
Infected, %	65.6	31.2	3.2	
IL1B rs16944	AA	GA	GG	.13
Noninfected, %	22.1	48.8	29.1	
Infected, %	31.8	38.5	29.7	
IL8 rs4073	AA	TA	TT	.66
Noninfected, %	56.5	36.5	7.1	
Infected, %	60.2	32.2	7.5	

[a] Includes 93 infected and 88 noninfected subjects.

of IL-10 upon stimulation with *C. albicans* conidia in PBMCs from individuals homozygous for the *IL10* rs1800896 G allele, compared with PBMCs from individuals homozygous for the A allele. Individuals heterozygous for this polymorphism exhibited an intermediate production of IL-10. The production capacity of PBMCs after stimulation with *C. albicans* hyphae was very low and revealed no differences between genotypes

Table 5. Univariate Analysis of Clinical, Microbiological, and Genetic Factors Associated With Persistent Fungemia in 298 Infected Adults at Duke University Medical Center[a]

Variable	Nonpersistent, % (n = 259)	Persistent, % (n = 39)	*P* Value
Female sex	42.3	45	.71
Immunocompromised	56.8	76.9	.02
HSCT	0.1	2.5	.31
Solid organ transplant	10.9	17.5	.23
Active malignancy[b]	31.8	27.5	.59
Solid tumor	22.5	15	.28
Leukemia	6.6	7.5	.74
Lymphoma	2.7	10.0	.05
Chemotherapy within past 3 months	15.9	22.5	.29
Neutropenia (ANC <500 cells/mm^3)	9.3	15	.26
HIV-infected	1.6	0	1.00
Surgery within past 30 days	43.2	45.0	.83
Receipt of total parenteral nutrition	17.0	32.5	.02
Dialysis dependent	10.7	23.1	.02
Acute renal failure	34.6	32.5	.79
Liver failure	24.5	27.5	.69
ICU admission within past 14 days	50.2	47.5	.75
Candida species			
albicans	43.0	35.0	.34
glabrata	29.5	27.5	.80
parapsilosis	14.0	27.5	.03
tropicalis	12.4	15.0	.65
krusei	3.5	5	.64
Other *Candida* species	3.5	0	.61
>1 *Candida* species	5.1	7.5	.46
Bacteremia within 48 hours	24.8	17.5	.31
Bacteremia within 14 days	34.9	27.5	.36
IFNG rs2430561 (AA)	12.2	12.5	1.00
IL10 rs1800872 (TT)	10.9	5.0	.39
IL10 rs1800896 (AA)	34.8	17.5	.03
IL12B rs41292470 (INS/INS)	24.3	7.7	.02
IL12B rs3212227 (CC)	7.5	2.6	.49
IL12RB1 rs17882232 (TT)	2.8	12.5	.01
IL12RB1 rs17884858 (GG)	7.6	12.8	.34
IL18 rs187238 (CC)	43.0	47.4	.62
IL18 rs549908 (GG)	7.8	7.7	1.00
IL1B rs1143634 (AA)	4.7	0	.38
IL1B rs16944 (AA)	20.6	12.5	.29
IL8 rs4073 (AA)	32.4	38.5	.44

Abbreviations: ANC, absolute neutrophil count; HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit.

[a] Variables with P < .2 were further assessed in multivariate analysis.

[b] Some patients had >1 active malignancy.

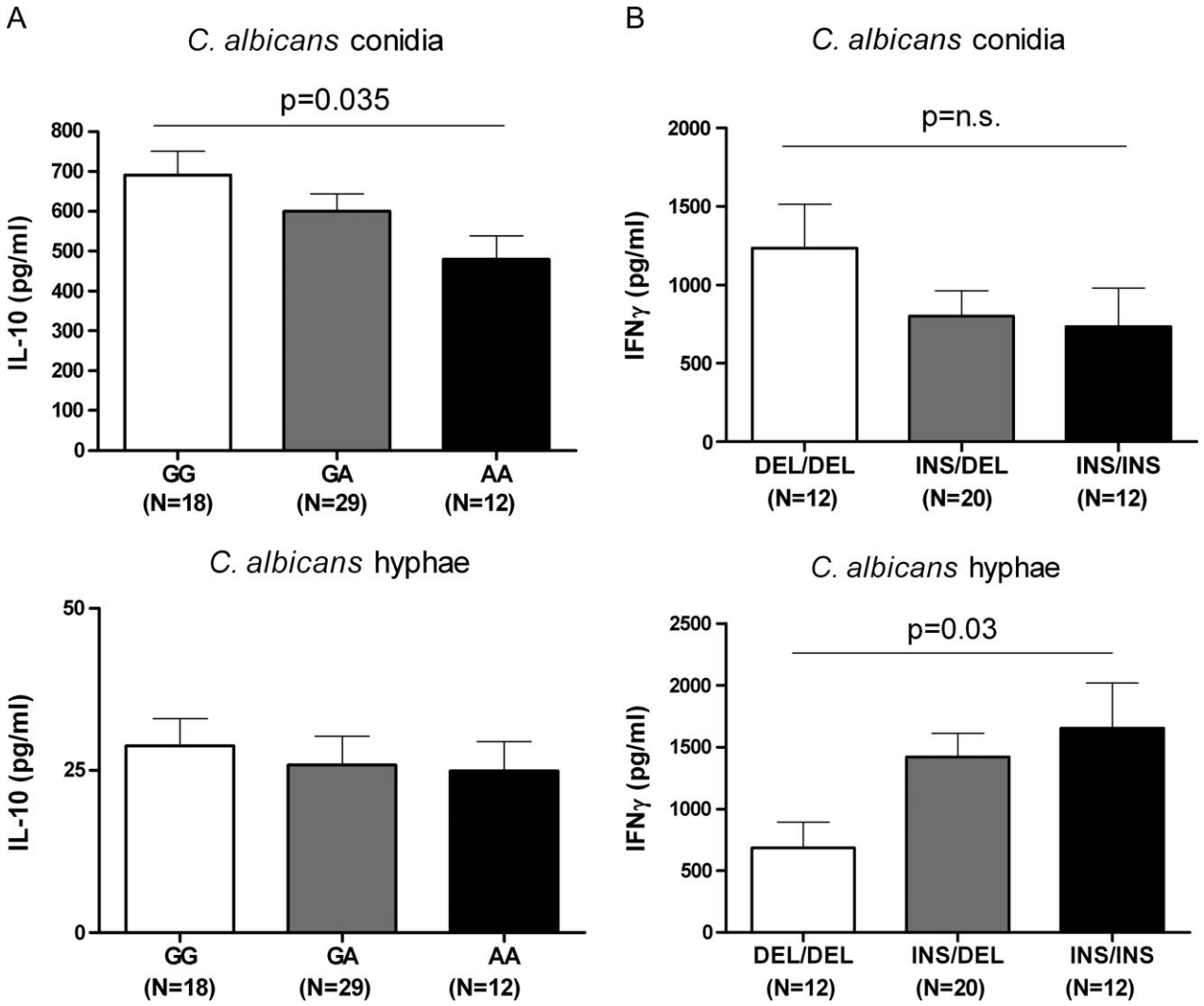

Figure 1. *IL10* and *IL12B* polymorphisms and *Candida*-induced cytokine responses. *A*, Cytokine production capacity of interleukin (IL)–10 of peripheral blood mononuclear cells (PBMCs) from healthy individuals with different *IL10* rs1800896 genotypes, unstimulated or stimulated with either 10^6/mL *C. albicans* conidia or 10^6/mL *C. albicans* hyphae for 48 hours. Data are mean ± standard error of mean (SEM). *B*, Cytokine production capacity of interferon-γ (IFN-γ) of PBMCs from healthy individuals with different *IL12B* rs41292470 genotypes, unstimulated or stimulated with either 10^6/mL *C. albicans* conidia or 10^6/mL *C. albicans* hyphae for 48 hours. Data are mean ± SEM.

(Figure 1*A*). When PBMCs were stimulated with these same stimuli and were stratified for their *IL12B* rs41292470 genotype, a 3-fold increased production of IFN-γ was observed by PBMCs homozygous for the insertion allele, compared with PBMCs from individuals homozygous for the *IL12B* deletion allele upon stimulation with *C. albicans* hyphae but not conidia. Again, PBMCs from heterozygous individuals produced intermediate amounts of IFN-γ (Figure 1*B*).

Circulating Cytokine Concentrations

Lower proinflammatory cytokine concentrations (IFN-γ, IL-8, IL-6) were apparent in serum/plasma from patients with persistent fungemia, compared with patients who cleared *Candida* rapidly. While the differences were statistically significant for IFN-γ and IL-8, the differences in circulating IL-6 concentrations were not statistically significant (Table 6, Mann-Whitney *U* test).

DISCUSSION

The interest in genetic susceptibility to systemic fungal infections has increased in recent years, being stimulated by the discovery of several polymorphisms in immune genes associated with invasive fungal infections such as aspergillosis [12, 13]. However, much less is known in patients with systemic *Candida* infections, and no systematic study has been dedicated to this most prevalent systemic fungal infection. To our knowledge, this

Table 6. Cytokine Concentrations of IFN-γ, IL-6, and IL-8 in Plasma and Serum Samples Obtained From Infected Patients From Day 0 to Day 2 After Initial Positive Blood Culture

	No Persistent Fungemia		Persistent Fungemia		
	No.	Mean (95% CI)	No.	Mean (95% CI)	*P* Value[a]
IFN-γ (pg/mL)					
Days 0–1	75	359 (103–615)	11	48 (20–77)	.01
Days 0–2	112	399 (105–694)	16	43 (19–66)	.01
IL-6 (pg/mL)					
Days 0–1	75	1174 (581–1768)	11	554 (137–970)	.08
Days 0–2	112	1296 (498–2094)	16	523 (25–1021)	.09
IL-8 (pg/mL)					
Days 0–1	75	1007 (421–1594)	11	299 (72–527)	.02
Days 0–2	112	1109 (408–1811)	16	252 (97–408)	.01

Abbreviations: CI, confidence interval; IFN-γ, interferon-γ; IL, interleukin.

[a] Mann-Whitney *U* test.

is the first large prospective study aimed at characterizing the role of SNPs in genes of the cytokine network along with other risk factors for susceptibility to invasive *Candida* infections.

The first observation made in this study is that SNPs in the cytokine genes, chosen on the basis of in vitro and clinical studies suggesting a functional role in infection, do not seem to impact the overall susceptibility to systemic *Candida* infections. This conclusion is supported by analysis of both patients with European and African ancestry. In addition, no differences in the *Candida* species were identified between patients with different cytokine polymorphisms, compared with those carrying wild-type alleles. It appears therefore that exogenous factors well known to impact susceptibility to systemic fungal infections (neutropenia, total parenteral nutrition, dialysis, intensive care unit stay) may supersede the impact of cytokine polymorphisms. Alternatively, polymorphisms in other important classes of innate immunity genes such as *TLR4*, *TLR2*, or *TLR1*, may play a more important role in the susceptibility to acquire the infection [14, 15].

In contrast to the conditions influencing susceptibility to infection, a different set of factors is likely to determine the progression of the disease. In sepsis patients, one of the major factors impacting the outcome of the infection is represented by development of immunoparalysis. Immunoparalysis is characterized by counterregulatory antiinflammatory responses, decreased expression of major histocompatibility complex class II (MHC-II), and lymphocyte apoptosis. A major factor influencing these processes is represented by the capacity of the host to mount a Th1/IFN-γ response, known to activate antifungal properties of neutrophils and macrophages and increase cell survival and MHC-II expression [16]. In contrast, Th2-type responses represented by the antiinflammatory cytokines IL-10 and IL-4 counteract the action of IFN-γ [17]. The importance of the Th1/Th2 balance for the outcome of systemic fungal infections has been demonstrated in series of elegant experimental models [18, 19].

These data in mouse models of candidiasis are now for the first time supported by our findings demonstrating that common polymorphisms in *IL12B* and *IL10* have a significant impact on the persistence of systemic *Candida* infections in humans. IL-12 is a cytokine secreted by activated phagocytes and dendritic cells that induces IFN-γ production by natural-killer and T lymphocytes. IL-12 consists of 2 subunits, p35 and p40, which are encoded by IL12A and IL12B, respectively. In previous studies, the presence of this *IL12B* SNP in the promoter region of the gene has been shown to be accompanied by higher production of IL-12 and IFN-γ [20]. In the present study we demonstrate that an insertion/deletion polymorphism in the promoter region of *IL12B* influences the likelihood of the patients to develop persistent infection with *Candida*. While the effect of the *IL12B* promoter polymorphism on IL-12 expression is complex and secretion of this cytokine in vivo may be influenced by several factors including IL-10, previous studies suggested that dendritic cells from individuals bearing the *IL12B* insertion allele produced the highest amounts of IL-12 upon stimulation [21]. This *IL12B* allele proved also to protect against persistent candidemia in our study, and this is in line with the beneficial effects of enhanced IL-12/Th1 responses in fungal infections.

In contrast, IL-10 counterbalances the effects of proinflammatory cytokines, including IFN-γ. The *IL10* SNP at –1082 (G to A) modifies IL-10 secretion and may influence outcome in several disease states [22]. The *IL10* –1082 G/A SNP lies within a putative ETS-consensus binding site and is associated (A allele) with lower IL-10 production [23, 24]. Hence, the *IL10* –1082 SNP may modify the response to sepsis caused by a variety of microorganisms [25]. The G allele in the promoter region of *IL10* at position –1082 was also associated with a higher rate of persistence of the infection, and in other studies individuals bearing the G allele had higher IL-10 production [26]. In the present study, this same G allele is also associated with a higher probability of developing persistent fungemia, which implies that high IL-10 production is predisposing to prolonged candidemia.

The results from the in vitro PBMC stimulation assays support the associations of the *IL10* and *IL12B* polymorphisms with persistent fungemia. The *IL10* G allele and the *IL12B* DEL allele, which both increase the risk for persistent fungemia, were demonstrated to modulate cytokine responses by PBMCs from healthy volunteers when stimulated with *C. albicans* conidia or hyphae; the *IL10* G allele is associated with higher production of IL-10 by PBMCs when stimulated with *C. albicans* conidia, whereas the *IL12B* DEL allele was shown to downmodulate

the production of IFN-γ specifically upon stimulation with *C. albicans* hyphae.

Unfortunately, detectable concentrations of IL-10 and IL-12 could not be measured in serum samples from our study patients, but our results are further supported by our analysis of other circulating cytokines, which demonstrate very low cytokine responses (IFN-γ and IL-8) in patients with persistent fungemia. The concentrations of these circulating cytokines could not be shown to be directly related to *IL12B* and *IL10* polymorphisms in our cohort, probably owing to the relatively small number of subjects in whom cytokines could be measured.

The important role that proficient Th1 responses play in severe systemic *Candida* infections is indirectly supported by the earlier observation that counterbalancing Th2/IL-10 responses are deleterious in patients with invasive aspergillosis [27]. Moreover, there is an accumulating body of evidence that treatment of patients with either invasive *Candida* [28] or *Aspergillus* [29] infections with recombinant proinflammatory cytokines such as IFN-γ, granulocyte macrophage colony-stimulating factor (CSF), or granulocyte CSF, has beneficial effects on the outcome of systemic fungal infections.

Other genes investigated (*IL1β*, *IL8*, *IL10*, *IL18*, *IFNG*, *IL12RB1*) did not reveal significant effects on susceptibility to systemic *Candida* infections or severity of the disease. In line with this lack of effect at the genetic level, cytokine production in in vitro PBMC stimulation assays and circulating concentrations of IFN-γ, IL-6, and IL-8 were not influenced by the presence of these polymorphisms (data not shown).

Apart from the association of *IL10* and *IL12B* polymorphisms, this study also revealed the association of persistent fungemia with total parenteral nutrition and dialysis dependence, which is consistent with previous studies [30]. Persistent fungemia is an increasingly recognized complication of *Candida* infections and has been reported in 8%–15% of candidemic patients in randomized controlled clinical trials.

In conclusion, in the present study we demonstrate that polymorphisms in genes modulating the Th1/Th2 responses (*IL12B* and *IL10*) influence the likelihood of systemic *Candida* infection persistence in a large cohort of patients. This study provides, to our knowledge, the first genetic markers that may be used to predict a prolonged course of disease and which need to be confirmed in future studies. Furthermore, the association of low Th1 responses with unfavorable outcome of infection provide additional arguments for adjunctive immunotherapy with recombinant IFN-γ in systemic *Candida* infections.

Supplementary data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Authors' contributions. T. S. P., E. v. d. V., D. K., and M. O. performed the experiments. E. v. d. V. and D. K. selected SNPs and designed the genotyping assays. M. D. J., P. B. S., B. D. A., J. C. Y., G. M. L., J. R. P., and M. G. N. managed the collection of the cohort. M. D. J., D. R. V. E., and W. K. S. performed the clinical statistical analysis. T. S. P., M. D. J., J. R. P., T. W., and M. G. N. designed the study and wrote the manuscript. All authors read and approved the final manuscript.

Financial support. This work was supported by a Vici grant from the Netherlands Organization for Scientific Research (to M. G. N.), and National Institutes of Health grants (AI-51537 to M. D. J. and AI-73896 to J. R. P.). All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Garey KW, Rege M, Pai MP, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis **2006**; 43:25–31.
2. Romani L. Immunity to fungal infections. Nat Rev Immunol **2004**; 4:1–23.
3. Netea MG, Brown GD, Kullberg BJ, Gow NA. An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat Rev Microbiol **2008**; 6:67–78.
4. Ashman RB, Farah CS, Wanasaengsakul S, Hu Y, Pang G, Clancy RL. Innate versus adaptive immunity in *Candida albicans* infection. Immunol Cell Biol **2004**; 82:196–204.
5. Romani L. Immunity to *Candida albicans*: Th1, Th2 cells and beyond. Curr Opin Microbiol **1999**; 2:363–7.
6. Tuite A, Mullick A, Gros P. Genetic analysis of innate immunity in resistance to *Candida albicans*. Genes Immun **2004**; 5:576–87.
7. Sørensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. N Engl J Med **1988**; 318:727–32.
8. Ferwerda G, Meyer-Wentrup F, Kullberg BJ, Netea MG, Adema GJ. Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. Cell Microbiol **2008**; 10:2058–66.
9. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet **2007**; 81:559–75.
10. Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics **1995**; 29:311–22.
11. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science **2002**; 296:2225–9.
12. Ferwerda B, Ferwerda G, Plantinga TS, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med **2009**; 361:1760–7.
13. Bochud PY, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. N Engl J Med **2008**; 359:1766–77.
14. Van der Graaf CA, Netea MG, Morré SA, et al. Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for *Candida* bloodstream infection. Eur Cytokine Netw **2006**; 17:29–34.
15. Woehrle T, Du W, Goetz A, et al. Pathogen specific cytokine release reveals an effect of TLR2 Arg753Gln during *Candida* sepsis in humans. Cytokine **2008**; 41:322–9.
16. Murray HW. Interferon-gamma in infection and immunoparalysis. Intensive Care Med **1996**; 22(Suppl 4):S455.

17. Puccetti P, Romani L, Bistoni F. A TH1-TH2-like switch in candidiasis: new perspectives for therapy. Trends Microbiol **1995**; 3:237–40.
18. Romani L, Mencacci A, Grohmann U, et al. Neutralizing antibody to interleukin 4 induces systemic protection and T helper type 1-associated immunity in murine candidiasis. J Exp Med **1992**; 176: 19–25.
19. Romani L, Puccetti P, Mencacci A, et al. Neutralization of IL-10 up-regulates nitric oxide production and protects susceptible mice from challenge with *Candida albicans*. J Immunol **1994**; 152: 3514–21.
20. Morahan G, Huang D, Wu M, et al. Association of IL12B promoter polymorphism with severity of atopic and non-atopic asthma in children. Lancet **2002**; 360:455–9.
21. Müller-Berghaus J, Kern K, Paschen A, et al. Deficient IL-12p70 secretion by dendritic cells based on IL12B promoter genotype. Genes Immun **2004**; 5:431–4.
22. Zeng L, Gu W, Chen K, et al. Clinical relevance of the interleukin 10 promoter polymorphisms in Chinese Han patients with major trauma: genetic association studies. Crit Care **2009**; 13:R188.
23. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet **1997**; 24:1–8.
24. Stanilova SA, Miteva LD, Karakolev ZT, Stefanov CS. Interleukin-10-1082 promoter polymorphism in association with cytokine production and sepsis susceptibility. Intensive Care Med **2006**; 32:260–6.
25. Schaaf BM, Boehmke F, Esnaashari H, et al. Pneumococcal septic shock is associated with the interleukin-10-1082 gene promoter polymorphism. Am J Respir Crit Care Med **2003**; 168:476–80.
26. Hunninghake GM, Soto-Quirós ME, Lasky-Su J, et al. Dust mite exposure modifies the effect of functional IL10 polymorphisms on allergy and asthma exacerbations. J Allergy Clin Immunol **2008**; 122:93–8.
27. Roilides E, Sein T, Roden M, Schaufele RL, Walsh TJ. Elevated serum concentrations of interleukin-10 in nonneutropenic patients with invasive aspergillosis. J Infect Dis **2001**; 183:518–20.
28. Bodasing N, Seaton RA, Shankland GS, Pithie A. Gamma-interferon treatment for resistant oropharyngeal candidiasis in an HIV-positive patient. J Antimicrob Chemother **2002**; 50:765–6.
29. Quezada G, Koshkina NV, Zweidler-McKay P, Zhou Z, Kontoyiannis DP, Kleinerman ES. Intranasal granulocyte-macrophage colony-stimulating factor reduces the *Aspergillus* burden in an immunosuppressed murine model of pulmonary aspergillosis. Antimicrob Agents Chemother **2008**; 52:716–8.
30. Kovacicova G, Lovaszova M, Hanzen J, et al. Persistent fungemia–risk factors and outcome in 40 episodes. J Chemother **2001**; 13:429–33.