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ARTICLE

The impact of caspase-12 on susceptibility to candidemia

D. C. Rosentul · T. S. Plantinga · W. K. Scott · B. D. Alexander · N. M. D. van de Geer · J. R. Perfect · B. J. Kullberg · M. D. Johnson · M. G. Netea

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Abstract *Candida* is one of the leading causes of sepsis, and an effective host immune response to *Candida* critically depends on the cytokines IL-1β and IL-18, which need caspase-1 cleavage to become bioactive. Caspase-12 has been suggested to inhibit caspase-1 activation and has been implicated as a susceptibility factor for bacterial sepsis. In populations of African descent, *CASPASE-12* is either functional or non-functional. Here, we have assessed the frequencies of both *CASPASE-12* alleles in an African-American *Candida* sepsis patients cohort compared to uninfected patients with similar predisposing factors. African-American *Candida* sepsis patients (n=93) and non-infected African-American patients (n=88) were genotyped for the *CASPASE-12* genotype. Serum cytokine concentrations of IL-6, IL-8, and IFNγ were measured in the serum of infected patients. Statistical comparisons were performed in order to assess the effect of the *CASPASE-12* genotype on susceptibility to candidemia and on serum cytokine concentrations. Our findings demonstrate that *CASPASE-12* does not influence the susceptibility to *Candida* sepsis, nor has any effect on the serum cytokine concentrations in *Candida* sepsis patients during the course of infection. Although the functional *CASPASE-12* allele has been suggested to increase susceptibility to bacterial sepsis, this could not be confirmed in our larger cohort of fungal sepsis patients.

D. C. Rosentul · T. S. Plantinga (✉) · N. M. D. van de Geer · B. J. Kullberg · M. G. Netea
Department of Medicine,
Radboud University Nijmegen Medical Centre,
Internal postal code 463, P.O. Box 9101, Geert Grooteplein 8,
6500 HB Nijmegen, The Netherlands
e-mail: t.plantinga@aig.umcn.nl

D. C. Rosentul · T. S. Plantinga · N. M. D. van de Geer · B. J. Kullberg · M. G. Netea
Nijmegen Institute for Infection,
Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Centre,
P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

W. K. Scott
Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics,
University of Miami, Miller School of Medicine,
Miami, FL 33136, USA

B. D. Alexander · J. R. Perfect · M. D. Johnson
Duke University Medical Centre,
Durham, NC 27708, USA

M. D. Johnson
Department of Clinical Research,
Campbell University School of Pharmacy,
Buies Creek, NC 27506, USA

Introduction

One of the leading pathogens causing sepsis in immunocompromised hosts are *Candida* spp. [1, 2]. Medical conditions that lead to an immunocompromised state increase susceptibility to *Candida* sepsis [3]. In addition to exogenous factors, it is believed that genetic variation also plays an important role in susceptibility to sepsis [4–6]. Caspase-12 is an inflammatory caspase, in which a loss-of-function genetic variant has been fixed in some populations by still undefined evolutionary pressures [7–9]. This loss-of-function is due to the presence of a T/C single nucleotide polymorphism (rs497116) on nucleotide position 125 in the *CASPASE-12* gene [10]. Although the ancestral variant is still present in African and African-American populations, of which 20–30% express the active

variant of caspase-12, it is absent in Asian and Caucasian populations [9, 10].

Functional studies have suggested that functional caspase-12 is a negative regulator of caspase-1 activation, which might result in less cytokine production in response to recognition through pattern recognition receptors. Thus, based on the proposed inhibitory effect on caspase-1 and, consequently, lower IL-1β and IL-18 production, functional caspase-12 may increase the susceptibility to severe sepsis and/or the clinical outcome of sepsis patients [10]. Therefore, it is compelling to assess whether genetic variation in *CASPASE-12* plays a role in the susceptibility to *Candida* sepsis. The aim of this study was to assess whether genetic variants of *CASPASE-12* influence the incidence, severity, and mortality of *Candida* sepsis in a cohort of African-American patients.

Patients, materials, and methods

Subjects were enrolled between January 2003 and January 2009 after informed consent (or waiver, as approved by the Institutional Review Board) at the Duke University Hospital (DUMC, Durham, NC, USA). Infected subjects had ≥1 positive blood cultures for a *Candida* species while hospitalized. Non-infected controls were recruited from the same hospital wards as infected patients, with no history or evidence of *Candida* sepsis/invasive candidiasis or any invasive fungal infection.

Genomic DNA was isolated from whole blood using standard procedures. The region of interest of the *CASPASE-12* gene was amplified as described previously [10].

Circulating cytokine concentrations of IL-6, IL-8, and IFNγ in infected patients were measured by Multiplex Fluorescent Bead Immunoassays (xMAP technology, Bio-Rad, Veenendaal, the Netherlands), from day 0 up to day 5 after the initial positive blood culture.

Statistical comparisons of frequencies were made between infected versus non-infected subjects using Chi-square tests. Statistical analysis of the cytokine data was performed by using the Mann–Whitney *U*-test. Overall, a p-value<0.05 was considered to be statistically significant.

Results

A total of 93 African-American patients and 88 non-infected African-American controls had genetic and clinical data available for the analysis. The demographic data for the study subjects are presented in Table 1.

No significant differences in the distribution of *CASPASE-12* genotypes were seen when comparing infected patients (CC 3.9%, CT 25.3%, TT 72.4%) and non-infected controls (CC 2.9%, CT 30.0%, TT 66.1%) ($p>0.05$). No associations between the *CASPASE-12* genotypes and disseminated disease, persistent fungemia, or 30-day mortality were observed (data not shown).

Serum samples collected from infected patients during the first 5 days after the initial positive blood culture were measured for concentrations of IL-6, IL-8, and IFNγ. Also, measurements of IL-1β and IL-18 were performed in these samples. However, the concentrations of these cytokines were too low to detect (data not shown). Cytokine concentrations decreased over time. No differences in

Table 1 Baseline patient characteristics of African-American patients with *Candida* systemic infection or uninfected controls recruited at the Duke University Hospital (DUMC, Durham, NC, USA) (n=181)

Variable	Infected cohort (n=93), %	Control cohort (n=88), %
Mean age (years)	52	52
Gender		
Male	51.6	48.9
Family	48.4	51.1
Immunocompromised state	54.8	48.9
HSCT	0	0
Solid organ transplant	7.5	3.5
Active malignancy*	22.6	13.8
Solid tumor	14	8.0
Leukemia	5.4	3.5
Lymphoma	3.2	2.3
Chemotherapy within past 3 months	12.9	5.6
Neutropenia (ANC <500 cells/mm^3)	4.3	1.2
HIV-infected	5.4	0
Surgery within past 30 days	34.4	30.7
Receipt of total parenteral nutrition	19.4	5.75
Dialysis-dependent	15.1	8.0
Acute renal failure	36.6	33.0
Liver failure	25.8	1.2
Intensive care unit admission within the past 14 days	39.8	31.8
Median baseline serum creatinine (mg/dL)	2.27	1.9
Median baseline WBC count (cells/mm^3)	13.0	10.87
Candida spp.**		
albicans	44.2	–
glabrata	23.7	
parapsilosis	17.2	
tropicalis	10.8	
krusei	3.2	
Other *Candida* spp.	0.9	

*Subjects could have more than one active malignancy

**Sixteen subjects had >1 species isolated

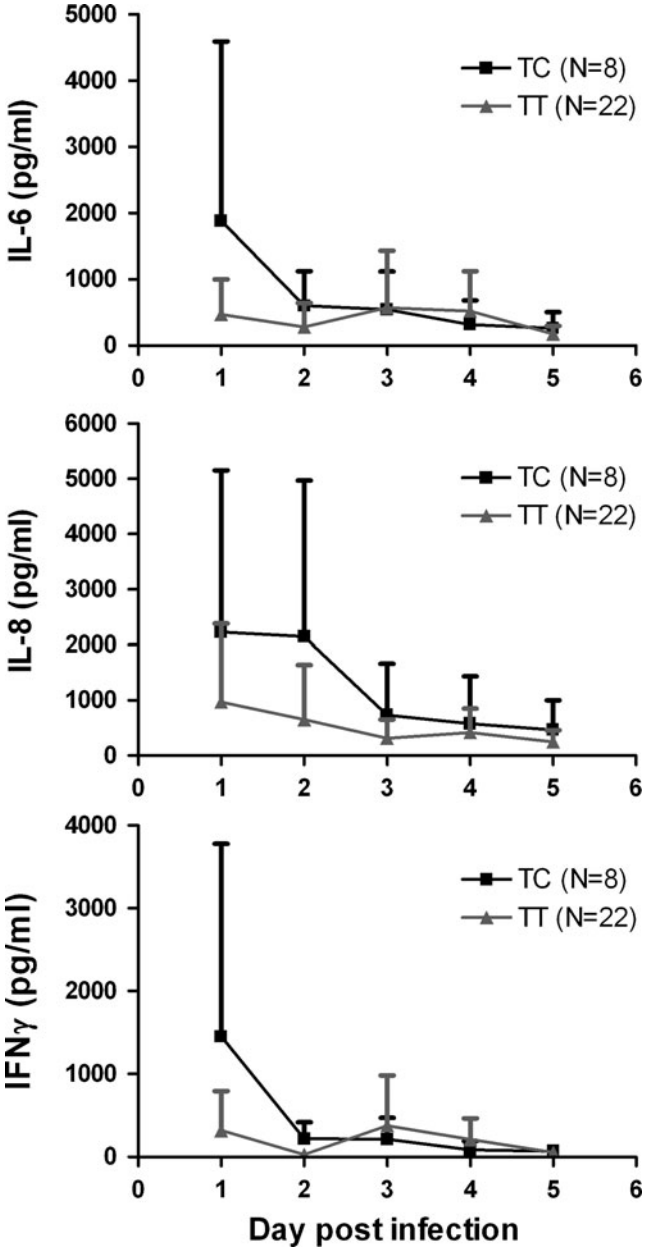

Fig. 1 IL-6, IL-8, and IFNγ circulating concentrations in infected patients from day 0 up to day 5 after initial positive blood culture, in relation to the *CASPASE-12* genotype. *TC* heterozygous, *TT* homozygous mutant. The data are presented as mean±standard error of the mean (SEM)

cytokine concentrations were apparent between individuals bearing different *CASPASE-12* genotypes (Fig. 1).

Discussion

Caspase-12 has been suggested to inhibit caspase-1 processing of proIL-1β and proIL-18 into the active cytokines. Genetic variation of *CASPASE-12* in populations of African descent has been previously associated with susceptibility to bacterial sepsis [10]. The present study was performed in order to assess the role of caspase-12 in sepsis caused by *Candida* spp. The results indicate that the *CASPASE-12* genotype has no significant effect on the susceptibility and severity of systemic infections with *Candida*.

Candida is one of the leading pathogens causing sepsis [2, 11, 12]. Pro-inflammatory cytokines such as IL-1β and IL-18 are a crucial factor in eliciting an effective immune response to eradicate the infection. A modulatory step in the production of these cytokines is exerted at the level of caspase-1, a protease that cleaves the pro-form of these cytokines into shorter bioactive proteins [13, 14]. It has previously been reported that *CASPASE-12* knockout mice were better capable of clearing both local and systemic bacterial infections compared to wild-type mice, through an improved inflammatory response [15]. The same authors described a similar effect of caspase-12 in patients with bacterial sepsis, with individuals bearing functional caspase-12 being more susceptible to this condition [10]. However, the role of *CASPASE-12* genetic variants in fungal sepsis has not been addressed so far.

Firstly, the comparison of *CASPASE-12* genotype frequencies in African-American patients with non-infected controls revealed no statistically significant differences. Secondly, no effects of the *CASPASE-12* genotype was observed in relation to the clinical outcome of infection, assessed as disseminated disease, persistent fungemia, and 30-day mortality. Furthermore, serum cytokine concentrations during the first 5 days of infection were shown to be unaffected by the *CASPASE-12* genotype.

Our findings on the lack of influence of the *CASPASE-12* genotype on fungal sepsis contrast with those of Saleh et al. [10, 15], who suggested an important role of this genetic variant in bacterial sepsis. Moreover, circulating cytokine concentrations in infected patients were also not influenced by the *CASPASE-12* genotype. It should be emphasized that this is, in particular, true for IL-6 and IFNγ, cytokines that are induced by IL-1β and IL-18, respectively [16–18]. This provides indirect evidence that functional caspase-12 has no clear effect on the production of IL-1β and IL-18 in the context of *Candida* sepsis. One possible explanation for the discrepancy between this study and that of Saleh et al. [10] is represented by the different cause of sepsis in the two studies, fungal and bacterial, respectively. However, one has to concede that the pro-inflammatory cytokines, of which production is reportedly regulated by the *CASPASE-12* genotype, exert similar protective effects in bacterial and fungal sepsis [18–21]. In this respect, a recent study has also failed to reproduce the inhibitory effects of the *CASPASE-12* genotype of lipopolysaccharide and Gram-

negative bacteria-induced cytokine production [22], bringing into question the biological activity of caspase-12.

In conclusion, although an effect of the *CASPASE-12* genotype on the susceptibility to bacterial sepsis has been previously reported in a small cohort of African-American patients [10], this could not be confirmed in our larger cohort of fungal sepsis patients. Furthermore, clinical outcome and in vivo cytokine responses were not influence by the *CASPASE-12* genotype. Therefore, we propose that caspase-12 is redundant for systemic host defense in sepsis.

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Conflict of interest All authors declare no conflicts of interest.

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