Role of the toll-like receptor 4 Asp299Gly polymorphism in susceptibility to Candida albicans infection [letter]

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Reply

To the Editor—My colleagues and I appreciate the comments of Drs. DeVincenzo and Buckingham [1] on our recently published article [2]. They question the lack of a relationship between the amount of virus shed and severity of illness in respiratory syncytial virus (RSV) disease. We created a carefully modulated illness score that included 16 ventilated patients. In addition to finding no relationship between the amount of virus shed and this summation of the severity of illness, we were struck by the highly variable virus load in both nasal and endotracheal secretions of the 16 ventilated patients.

As Drs. DeVincenzo and Buckingham are aware [1], no therapeutic effect of RSV intravenous immune globulin could be demonstrated in the multicenter study from which these patients were drawn. The same was true in the subset of patients we studied at Vanderbilt University Medical Center (Nashville). Hence, we do not feel that this was a confounding variable. Perhaps neither their study [3] nor ours [2] provides a definitive answer to the presence of a modest effect of virus titer on illness severity, especially since neither study can address the peak titer of viral shedding that probably occurs before hospitalization. Both studies suggest that the effect, if any, of virus load on illness severity is modest and that we need to search elsewhere for a complete understanding of RSV pathogenesis.

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Role of the Toll-Like Receptor 4 Asp299Gly Polymorphism in Susceptibility to *Candida albicans* Infection

To the Editor—Despite the importance of *Candida albicans* in causing human disease, little is known of how it is recognized by cells and how the innate immune system is triggered to mount an effective defense. Recently, Netea et al. [1] reported that toll-like receptor 4 (TLR4)–defective C3H/HeJ mice [2] have an increased susceptibility to disseminated candidiasis (i.e., a 10-fold greater outgrowth of *C. albicans*). TLRs, a new class of pattern-recognition receptors, activate signaling pathways that induce antimicrobial responses and trigger inflammation, after recognition of pathogen-associated molecular patterns, and play a central role in innate immunity [3–6]. The TLR4 Pro712His polymorphism, which results in a defective gene in C3H/HeJ mice, is not present in humans [7]. However, the recently described TLR4 Asp299Gly polymorphism, which is found in humans, results in an endotoxine hyporesponsiveness in humans, which is similar to the phenotype of C3H/HeJ mice [8]. In addition, transfection of the human monocytic cell line THP-1 demonstrated that only the Asp299Gly polymorphism, not the Thr399Ile polymorphism, interrupts TLR4-mediated lipopolysaccharide signaling [9]. Finally, the Asp299Gly polymorphism may also predispose people to develop septic shock with gram-negative microorganisms, as recently reported elsewhere [10].

To further investigate the results reported by Netea et al. [1] concerning increased susceptibility to disseminated candidiasis in TLR4-defective C3H/HeJ mice, we investigated whether the TLR4 Asp299Gly polymorphism in humans would also be associated with increased susceptibility to and severity of *C. albicans* infection. We studied 222 white Dutch women <30 years old who visited the Sexually Transmitted Disease Outpatient Clinic of the Municipal Health Service of Amsterdam. The women were asked to complete a questionnaire regarding their symptoms, to divide them into symptomatic or asymptomatic groups. Cervical swabs were tested by culture for the presence of *C. albicans*. In addition, we tested for the presence of *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis,* herpes simplex virus (HSV)–1, and HSV-2, to correct for coinfection with regard to symptom presentation.

We developed a polymerase chain reaction (PCR)–based restriction fragment–length polymorphism assay to detect the A→G missense mutation at 896 bp (Asp299Gly) in the human

Table 1. Genotype frequencies of the toll-like receptor 4 Asp299Gly polymorphism in white Dutch women with and without *Candida albicans* infection, by their urogenital symptoms and coinfection status.

	Women with coinfection, by genotype				Women without coinfection, by genotype			
C. albicans infection status, presence of symptoms	Total no. of women	1.1	1.2	2.2	Total no. of women	1.1	1.2	2.2
Positive								
Symptomatic	49	43 (87.8)	6 (12.2)	0 (0)	45	39 (86.7)	6 (13.3)	0 (0)
Asymptomatic	39	35 (89.7)	3 (7.7)	1 (2.6)	33	29 (87.9)	3 (9.0)	1 (3.0)
Total	88	78 (88.6)	9 (10.2)	1 (1.1)	78	68 (87.2)	9 (11.5)	1 (1.3)
Negative								
Symptomatic	76	69 (90.8)	6 (7.9)	1 (1.3)	71	64 (90.1)	6 (8.5)	1 (1.4)
Asymptomatic	58	46 (79.3)	12 (20.7)	0 (0)	56	44 (78.5)	12 (21.4)	0 (0)
Total	134	115 (85.8)	18 (13.4)	1 (0.7)	127	108 (85.0)	18 (14.2)	1 (0.8)

NOTE. Data are no. (%) of women with the indicated genotype.

tlr4 gene (GenBank accession number U88880). PCR products of 102 bp (primers 5′-AGCATACTTAGACTACTACCTC-CATG-3′ and 5′-TTTACCCTTTCAATAGTCACACTCA-3′) were digested with *Nco*I (Invitrogen) and separated on a 4.5% agarose gel. Fragments of 102 bp (allele 1, wild type, *896A*) and/or 80 and 22 bp (allele 2, mutant, *896G*) were obtained. Fisher's exact or χ^2 test was used to compare genotype frequencies for case patients and control subjects. Logistic regression analysis was applied to fit statistical models, including *C. albicans* positivity, symptoms, and coinfection.

As shown by the genotype frequencies of the TLR4 Asp299Gly polymorphism (table 1), no difference in susceptibility to infection between *C. albicans*–positive ($n = 88$) and –negative ($n = 134$) white Dutch women was observed. In addition, the presence of symptoms was not associated with the analyzed mutation. Finally, correction for coinfections to better validate the presence of symptoms did not change the genotype frequencies (table 1).

These results indicate that the functional TLR4 mutation analyzed does not seem to play a role in the susceptibility to and severity of human urogenital *C. albicans* infection. This might imply that there is a different role for TLR4 in human host defense against *C. albicans,* compared with the experimental model of disseminated candidiasis in mice. As shown by Netea et al. [1], the production of interleukin-8, a CXC chemokine considered to be a human homologue of murine KC, and macrophage inhibitory protein (MIP)–2 was not influenced by blocking TLR4, whereas, in mice, blocking of TLR4 correlated with a defective production of the neutrophil chemokines KC and MIP-2. It would be interesting to further analyze the role of TLRs and their coreceptors in relation to the genotypes for these receptors for the induction of cytokines and chemokines by *C. albicans* in peripheral blood mononuclear cells while including patients of different ethnic backgrounds with acute disseminated candidiasis or symptomatic urogenital candidiasis.

The recent discovery of TLRs, which enable the innate immune system to detect the presence and the nature of infection, providing the first line of host defense and controlling the initiation and determination of the effector class of the adaptive immune response, has enabled new ways to study the host defense against a broad range of microbial infections. Several unknown factors are still to be unveiled regarding the innate immune system and its signal routes. However, it is clear that a systematic approach to the study of innate immunity to the common *C. albicans* infection will result in a better insight of its pathogenesis and control.

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All participants gave written informed consent.

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Reply

To the Editor—We read with interest the letter by Morré et al. [1] in which they report the results of their investigation of the toll-like receptor 4 Asp299Gly polymorphism in women with vaginal cultures positive for *Candida albicans*. The fact that they could not find an association between culture positivity and this polymorphism does not surprise us and does not contradict our previously published findings [2].

First, the polymorphism that was studied by Morré et al. [1] is one that has been shown to be relevant in the response to lipopolysaccharide of gram-negative microorganisms. It is, therefore, questionable whether it could be expected that this polymorphism would be relevant for the response to a yeast, such as *C. albicans*.

Second, we are concerned about the definition of vaginal candidiasis in the setting of a sexually transmitted diseases clinic. Positive cultures for *Candida* species, even for patients with symptoms, may reflect colonization, rather than infection.

Finally, in host defense against mucosal candidiasis, T lymphocytes are the major players, which is fundamentally different from the defense against disseminated candidiasis, which was the focus of our article [2], and is merely a function of phagocytic cells.

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