

An Exaggerated Monocyte-Derived Cytokine Response to *Candida* Hyphae in Patients With Recurrent Vulvovaginal Candidiasis

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Background. Recurrent vulvovaginal candidiasis (RVVC) affects up to 8% of women. The immunopathogenesis is poorly understood but it has been suggested that RVVC might be due to dysregulated innate immune response. The aim of this study was to compare cytokine profiles in stimulated primary mononuclear cells (PBMCs) from RVVC and healthy individuals.

Methods. PBMCs isolated from RVVC patients (n = 24) and healthy volunteers (n = 30) were stimulated with unspecific and pathogen-specific antigens. Cytokine production was assessed after 24 hours, 48 hours, and 7 days using ELISA.

Results. No significant differences in cytokine production were found in T helper 1 (Th1), Th2, and Th17 immunity in response to both unspecific and pathogen-specific stimulations. Tumor necrosis factor- α (TNF- α) production in response to *C. albicans* hyphae was significantly higher in patients than controls and within the patient group, a significant positive correlation was found between interleukin-1 β (IL-1 β) and both TNF- α and IL-6. Both IL-1 β /IL-1Ra and TNF- α /IL-10 ratios in *Candida* hyphae-stimulated PBMCs were significantly higher in patients than controls.

Conclusions. Women affected by RVVC showed increased monocytes-derived cytokine production, which might contribute to an exaggerated vaginal immune response to *Candida* hyphae. RVVC patients show no defective Th-dependent adaptive immune response upon *Candida* stimulation.

Keywords. recurrent vulvovaginal candidiasis; RVVC; *Candida albicans*; immune response; innate immunity; cytokines.

Vulvovaginal candidiasis (VVC) is a debilitating condition, affecting 75% of women of childbearing age globally every year [1]. Although *Candida* infections at the vaginal mucosa are not life-threatening, they strongly affect quality of life [2]. Women experiencing more than 3 episodes annually are classified as having recurrent VVC (RVVC) [3]. Because no vaccines are currently available, antifungal formulations are the only therapeutic options, although short courses do not prevent recurrences [4]. Host-related risk factors, such as diabetes mellitus, pregnancy, oral contraceptives usage, and immunosuppressive and antibiotic therapy, are associated with RVVC [5, 6]. However, the aforementioned predisposing factors are not

present in the majority of the patients and, so far, no sole causative agent has been found to be responsible for (R)VVC onset. To date, the immunopathogenesis of (R)VVC is poorly understood. As a higher incidence of mucosal *Candida* infections has been found in patients with reduced cell-mediated immunity (CMI) [7], a dysregulated adaptive immunity in response to *Candida* has been hypothesized to be involved in RVVC susceptibility. However, studies from both human and mouse models failed to identify a role for either systemic or local CMI deficiency (reviewed in [8]). An exacerbated innate immune response, particularly due to IL-1 β hyperproduction and polymorphonuclear neutrophils (PMNs) dysfunction, has been proposed to be responsible for RVVC immunopathogenesis [9, 10].

The purpose of the current study was to investigate the immune response in RVVC patients and healthy individuals. Thus, we assessed both adaptive and innate cytokine production capacity of peripheral blood mononuclear cells (PBMCs) challenged with both unspecific and pathogen-specific antigens.

METHODS

Patient Selection and Healthy Volunteers' Recruitment

Patients and healthy volunteers were recruited at Radboud University Medical Center (Nijmegen, the Netherlands). We included 24 consecutive women with RVVC. RVVC was

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defined as an episode with typical symptoms and vulvovaginal signs, with at least 3 reported episodes in the previous or following year and at least 1 episode in which microbiological culture was confirmed. Immunocompromised women were excluded. Inclusion of healthy controls was approved by the local institutional review board (region Arnhem-Nijmegen, number 2299 2010/104). According to the Dutch law, the patient study was exempt from approval by an ethics committee because the blood samples were part of regular care.

Isolation and Stimulation of Peripheral Blood Mononuclear Cells

For the purpose of the study, we isolated PBMCs, which are a heterogeneous mixed population consisting of lymphoid cells (T cells, B cells, and natural killer [NK] cells) and myeloid (monocytes and dendritic cells) cells. PBMCs are a useful and easily accessible material to investigate both the early cytokine production by monocytes and the later adaptive response by T-helper cells (Th-1, Th-2, and Th-17) against intruding pathogens, such as *Candida albicans*. PBMCs were isolated from EDTA blood by Ficoll-Paque density gradient centrifugation as described previously [11]. The PBMCs of at least 1 control was included in the same experiment. PBMCs were stimulated with RPMI (negative control), *Escherichia coli* lipopolysaccharide (LPS; 10 ng/mL), β -glucan (10 μ g/mL), LPS plus muramyl dipeptide (5 μ g/mL), phytoemagglutinin (10 μ g/mL), *C. albicans* UC820 (American Type Culture Collection [ATCC] MYA-3573) yeast heat-killed (HK) (1×10^5 cells/mL), *C. albicans* UC820 (ATCC MYA-3573) hyphae HK (1×10^6 cells/mL), and *C. albicans* UC820 (ATCC MYA-3573) yeast live (1×10^5 cells/mL) at 37°C for 24 hours, 48 hours, and 7 days (in the presence of 10% serum with the culture medium). Cell culture supernatants were collected and stored at -20°C until used for enzyme-linked immunosorbent assay (ELISA).

In particular, to investigate the Th17 immune response, interleukin (IL)-17 and IL-22 were measured after 7 days' stimulation. To elicit an effective protection against *Candida* infection, a balance in Th1 and Th2 response is crucial [7]. To test whether the Th1/Th2 function was impaired in RVVC patients, PBMCs were incubated for 48 hours with both unspecific and pathogen-specific stimuli, followed by measurements of interferon- γ (IFN- γ) and IL-10 levels. The innate immune response was investigated by the capacity of *C. albicans* yeast and hyphae to induce tumor necrosis factor- α (TNF- α), IL-6, IL-1 β , and IL-1Ra in PBMCs. Cytokine concentrations were determined using commercial ELISA kits for TNF- α , IL-6, IL-1 β , IL-1Ra, IL-17, IL-22 (R&D systems), IL-10, and IFN- γ (Sanquin Research) following the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean \pm standard error of the mean (SEM). The Mann-Whitney *U* test was used for the comparison of cytokine concentrations between the 2 independent groups.

The healthy control group included both female and male subjects. No differences in cytokine production were found between men and women (Supplementary Figure 1A). In addition, we did not find a relevant influence of control volunteers' age on cytokine production in line with previous findings [12] (Supplementary Figure 1B). For the assessment of correlations Spearman rank correlation coefficient was calculated. A *P* value of $< .05$ was considered statistically significant. Generation of graphs and statistical analyses were performed using GraphPad Prism 5.

RESULTS

Patient Characteristics

Twenty-four RVVC patients and 30 healthy volunteers were included in the study and their clinical characteristics are presented in Table 1. Briefly, 19 patients (79.1%) were using systemic hormonal contraception and 2 (8.3%) were taking other medication at the time of diagnosis. Fourteen patients were diagnosed with atopy (58.8%) and 7 patients (29.1%) reported other previous vaginal infections.

Normal Dectin-1 Signaling

Because the β -glucan receptor dectin-1, together with Toll-like receptor-2 (TLR-2) and TLR-4, plays a central role in *Candida* recognition and cytokine production [13], we checked whether dectin-1 signaling was defective. To do this, we compared IL-6 production of PBMCs after exposure to LPS (TLR4 ligand), β -glucan (dectin-1 ligand), and the combination of both. As illustrated in Supplementary Figure 2, overall, we did not find any significant alteration in dectin-1 signaling in the RVVC group; however, PBMCs from 3 of the 24 RVVC patients showed no synergism between β -glucan and LPS stimulation (as was present in all controls), which may indicate a partial defect in dectin-1 pathway.

Th1, Th2, and Th17 Function in RVVC

Neither unspecific nor pathogen-specific stimulations showed significant differences in IL-17 (Figure 1A) and IL-22 (Figure 1B) production levels, between PBMCs from patients and controls, except for a significant increase in IL-22 production in patients upon LPS plus β -glucan stimulation (Figure 1B; $P = .0109$). Focusing on the patient group, we next investigated the interrelationship between IL-17 and IL-22 levels in RVVC patients and found a significant positive correlation between IL-17 and IL-22 levels in both yeast-stimulated (Figure 1C; $r = 0.6337$, $P = .0004$) and hyphae-stimulated (Figure 1D; $r = 0.7161$, $P < .0001$) PBMCs.

Overall, PBMCs from RVVC patients displayed a similar or lower trend for IL-10 production when compared to controls (Figure 2A). Similarly, no significant differences in IFN- γ release (Figure 2B) were observed between the 2 groups. Taken together, these findings suggest a normal Th1/Th2 function in RVVC patients.

Table 1. Clinical Characteristics of RVVC Patients and Healthy Volunteers Included in the Study

Characteristic	RVVC (n = 24)		Control (n = 30)	
	Value	95% CI	Value	95% CI
Sex (M/F)	0/24		13/17	
Age, y, mean (± SD)	30.00 (± 8.827)	26.5–33.5	42.41 (± 17.36)	36.1–48.7
Atopy, No. (%)	14 (58.8)		NA	
Systemic hormonal contraception, No. (%)	19 (79.1)		NA	
Other medication usage, No. (%)	2 (8.3)		NA	
Previous vaginal infections, No. (%)	7 (29.1)		NA	
<i>Candida</i> species, No. (%)				
<i>C. albicans</i>	19 (79.1)		NA	
Unspecified	2 (8.3)		NA	
<i>C. dubliniensis</i>	1 (4.1)		NA	
<i>C. glabrata, C. lusitanae</i>	1 (4.1)		NA	
<i>C. famata, C. parapsilosis</i>	1 (4.1)		NA	
Blood count				
Leukocytes (×10 ⁶ /mL)	7.126 (± 1.44)	6.50–7.75	...	
Neutrophils (×10 ⁶ /mL)	4.314 (± 1.21)	3.78–4.85	...	
Lymphocytes (×10 ³ /mL)	2.097 (± 0.43)	1.91–2.283	...	
Monocytes (×10 ⁶ /mL)	0.5123 (± 0.10)	.47–.56	...	
Eosinophils (×10 ⁶ /mL)	0.1427 (± 0.11)	.093–.19	...	
Basophils (×10 ⁶ /mL)	0.035 (± 0.02)	.03–.04	...	
Mannose binding lectin, mg/L, reference 1.0–5.5	2.064	1.325–2.803	...	

Abbreviations: CI, confidence interval; RVVC, recurrent vulvovaginal candidiasis.

Innate Immunity: Exaggerated Cytokine Response to *Candida* Hyphae

TNF- α levels in PBMCs from RVVC patients in response to *Candida* hyphae were statistically significantly higher than controls ($P = .0013$). IL-6 and IL-1 β production by PBMC of RVVC patients did not differ from that in controls, whereas IL-1Ra concentrations tended to be lower in patients than in controls (Figure 3A). Conversely, monocytes-derived cytokine production in response to *C. albicans* yeast stimulation was similar in patients compared to controls (Supplementary Figure 3). *C. albicans* hyphae-induced IL-1 β levels in PBMCs from RVVC patients were positively correlated with both TNF- α ($r = 0.6045$, $P = .0009$) and IL-6 ($r = 0.5414$, $P = .0031$), as shown in Figure 3B. Similar results were found in yeast-stimulated PBMCs from RVVC patients (Supplementary Figure 4; TNF- α , $r = 0.7104$, $P < .0001$; IL-6, $r = 0.5671$, $P = .0019$).

Unbalanced Proinflammatory Response

After *Candida* yeast stimulation, we observed a positive correlation between IL-1 β and IL-1Ra in PBMCs from patients (Figure 4A; $r = 0.5322$, $P = .0037$), indicating that the IL-1 β production was adequately counterbalanced by IL-1Ra levels; accordingly, the IL-1 β /IL-1Ra ratio, reflecting IL-1 bioactivity, was not significantly different between controls and patients (Figure 4A, right). On the contrary, IL-1 β and IL-1Ra levels in hyphae-stimulated PBMCs from RVVC patients were not significantly correlated (Figure 4B left) and the IL-1 β /IL-1Ra ratio was significantly higher in patients compared to controls

(Figure 4B right; $P = .0169$). Moreover, the TNF- α /IL-10 ratio between controls and patients resulted in significantly higher TNF- α production in PBMCs from RVVC patients only upon stimulation with *Candida* hyphae (Figure 4C right; $P = .0015$).

To investigate a possible role for atopy in RVVC pathogenesis, as previously suggested [14], we stratified the RVVC group into 2 clinical subgroups based on atopic constitution and compared cytokine production capacity between the 2 subgroups and the healthy controls. We observed a trend towards higher production of proinflammatory cytokines upon challenge with *Candida* yeast and hyphae, with significantly higher TNF- α levels in *Candida* hyphae-stimulated PBMCs (Figure 5A; $P = .0066$). Furthermore, IL-1 β /IL-1Ra and TNF- α /IL-10 ratios were higher in atopic individuals when compared with the control and nonatopic groups upon *Candida* hyphae stimulation (Figure 5B; $P = .0014$ and $P = .0345$, respectively).

DISCUSSION

Our cohort of women with idiopathic RVVC was characterized by an inappropriately high proinflammatory innate cytokine response to *Candida* hyphae. Firstly, PBMCs from women with RVVC displayed an increased TNF- α production. Secondly, PBMCs from women with RVVC also showed a substantial increase of IL-1 β bioactivity compared to controls, upon stimulation with *Candida* hyphae, especially due to relatively low IL-1Ra concentrations. In contrast, we found no evidence of defective or altered Th1, Th2, or Th17 function. Cumulatively,

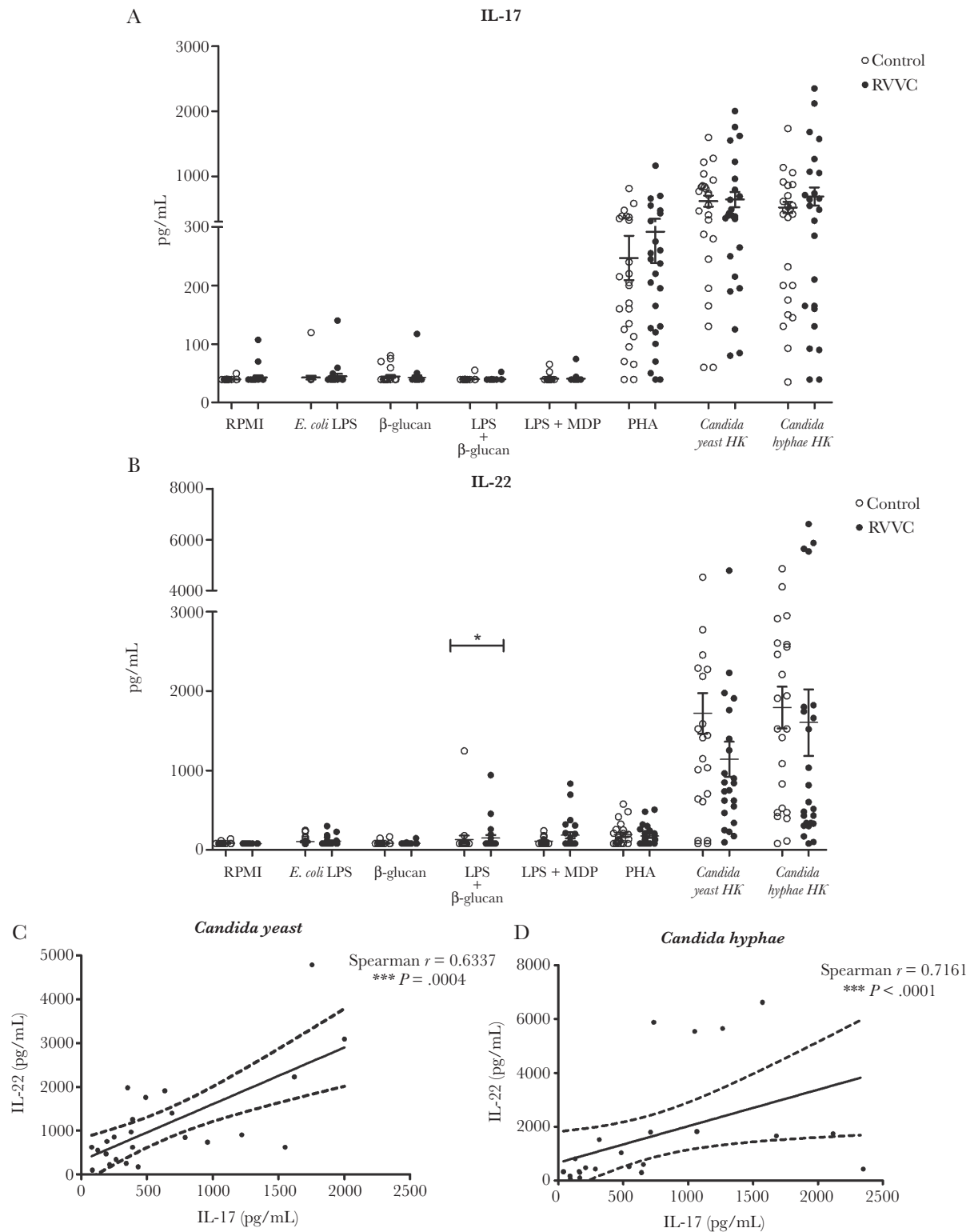


Figure 1. Th-17 response in PBMCs from RVVC patients and controls. PBMCs from healthy controls and RVVC patients were stimulated with RPMI, *Escherichia coli* LPS (10 ng/mL), β -glucan (10 μ g/mL), LPS plus β -glucan, LPS plus MDP (5 μ g/mL), PHA (10 μ g/mL), *Candida albicans* UC820 yeast HK (1×10^5 cells/mL), or *C. albicans* UC820 hyphae HK (1×10^6 cells/mL) for 7 days in the presence of 10% serum at 37 °C. IL-17 (A) and IL-22 (B) levels in culture supernatants were measured by ELISA. Results are means \pm SEM. Significance was determined with Mann-Whitney *U* test. * *P* value < .05 was considered statistically significant. Correlation within patients of IL-17 and IL-22 levels upon *C. albicans* yeast (UC820, HK) (C) and hyphae (UC820, HK) (D) stimulation of PBMCs. Correlation were analyzed using Spearman rank correlation coefficient test. Abbreviations: ELISA, enzyme-linked immunosorbent assay; HK, heat killed; IL, interleukin; LPS, lipopolysaccharide; MDP, muramyl dipeptide; PBMC, peripheral blood mononuclear cell; PHA, phytoemagglutinin; RVVC, recurrent vulvovaginal candidiasis; SEM, standard error of the mean.

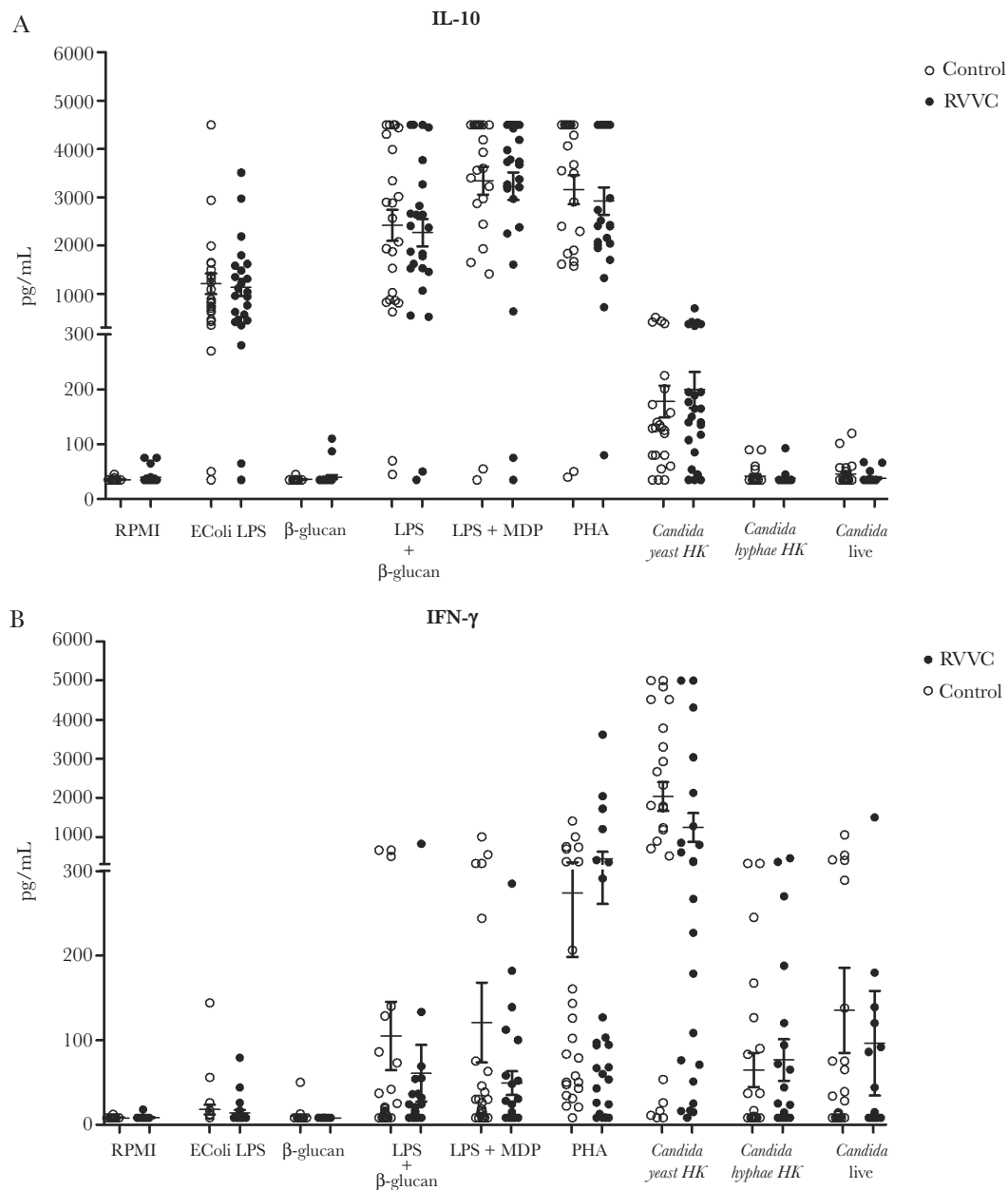


Figure 2. Th-1 and Th-2 response in PBMCs from RVVC patients and controls. PBMCs from healthy controls and RVVC patients were stimulated with RPMI, *Escherichia coli* LPS (10 ng/mL), β -glucan (10 μ g/mL), LPS plus β -glucan, LPS plus MDP (5 μ g/mL), PHA (10 μ g/mL), *Candida albicans* UC820 yeast HK (1×10^5 cells/mL), *C. albicans* UC820 hyphae HK (1×10^6 cells/mL), or *C. albicans* yeast UC820 live (1×10^5 cells/mL) for 48 hours at 37°C. IL-10 (A) and IFN- γ (B) levels were measured by ELISA in culture supernatants. Results are means \pm SEM. Significance was determined with Mann-Whitney *U* test. Abbreviations: ELISA, enzyme-linked immunosorbent assay; HK, heat killed; IL, interleukin; IFN- γ , interferon- γ ; LPS, lipopolysaccharide; MDP, muramyl dipeptide; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; RVVC, recurrent vulvovaginal candidiasis; SEM, standard error of the mean; Th, T helper cell.

these findings support the hypothesis that RVVC is likely the result of a vaginal immune exuberant response to *Candida* infection, similar to localized autoinflammatory diseases such as gout or hidradenitis suppurativa.

Our main findings are in line with mouse and clinical studies suggesting RVVC is the result of an acute local innate immune response driven by a positive feedback loop mechanism characterized by an exaggerated

IL-1 β production via NLRP3 inflammasome and failure of fungal clearance by infiltrated, but ineffective, PMNs [10]. Current pharmacologic treatment of RVVC consists solely of antifungal prophylaxis. Long-term weekly fluconazole effectively reduces recurrences, although approximately 10% of women have breakthrough infections [15]. More importantly, half of women relapse after cessation of fluconazole prophylaxis. Our findings offer a

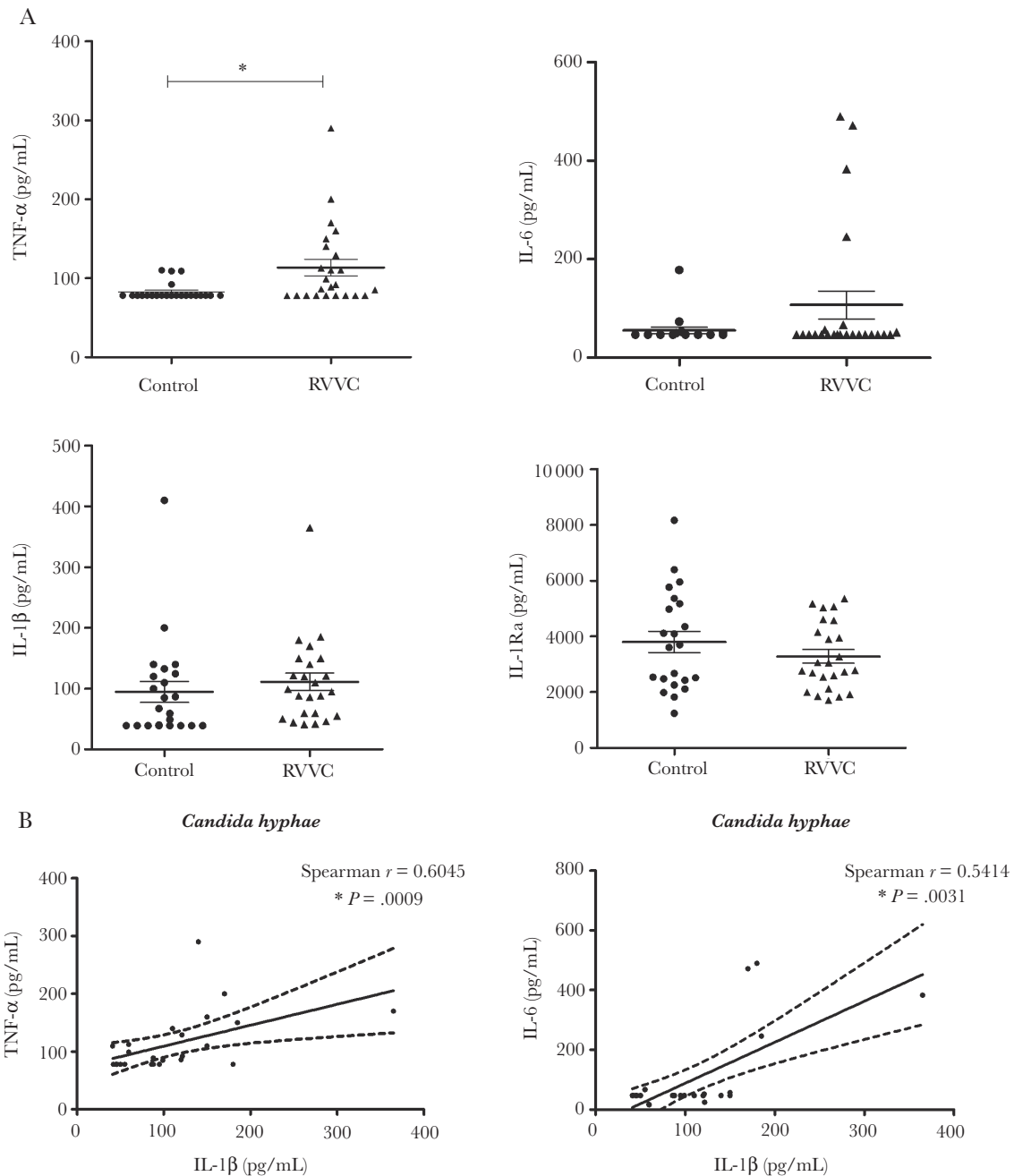


Figure 3. Innate response to *Candida albicans* hyphae. Cytokine induction in PBMCs after 24 hours stimulation with *C. albicans* hyphae HK (UC820, 1×10^6 cells/mL). *A*, TNF- α , IL-6, IL-1 β , and IL-1Ra levels were quantified in culture supernatants by ELISA. Results are means \pm SEM. Significance was determined with Mann-Whitney *U* test. *B*, Correlation of cytokines in hyphae-stimulated PBMCs within RVVC patients. Correlations were analyzed using Spearman rank correlation coefficient test. * *P* value < .05 was considered statistically significant. Abbreviations: ELISA, enzyme-linked immunosorbent assay; HK, heat killed; IL, interleukin; IL-1Ra, interleukin-1 receptor a; PBMC, peripheral blood mononuclear cell; RVVC, recurrent vulvovaginal candidiasis; SEM, standard error of the mean; TNF- α , tumor necrosis factor- α .

scientific base for a completely new therapeutic approach for RVVC, targeting of the underlying autoinflammatory state. IL-1 β is a pivotal cytokine in many autoinflammatory diseases and blocking its effect, for example by the IL-1 receptor antagonist anakinra, has been reported to be very effective [16]. Hence, similar approaches should be tested in RVVC.

In addition to dysregulated IL-1 activity, we investigated whether RVVC individuals display a defective T helper function. Indeed, defective Th17 has been reported to be one of the main causes of other mucosal *Candida* infections (eg, oral and esophageal). However, we did not observe a defective Th1/Th2/Th17 response in RVVC patients, strengthening the hypothesis that deficiencies in adaptive immunity are not involved in

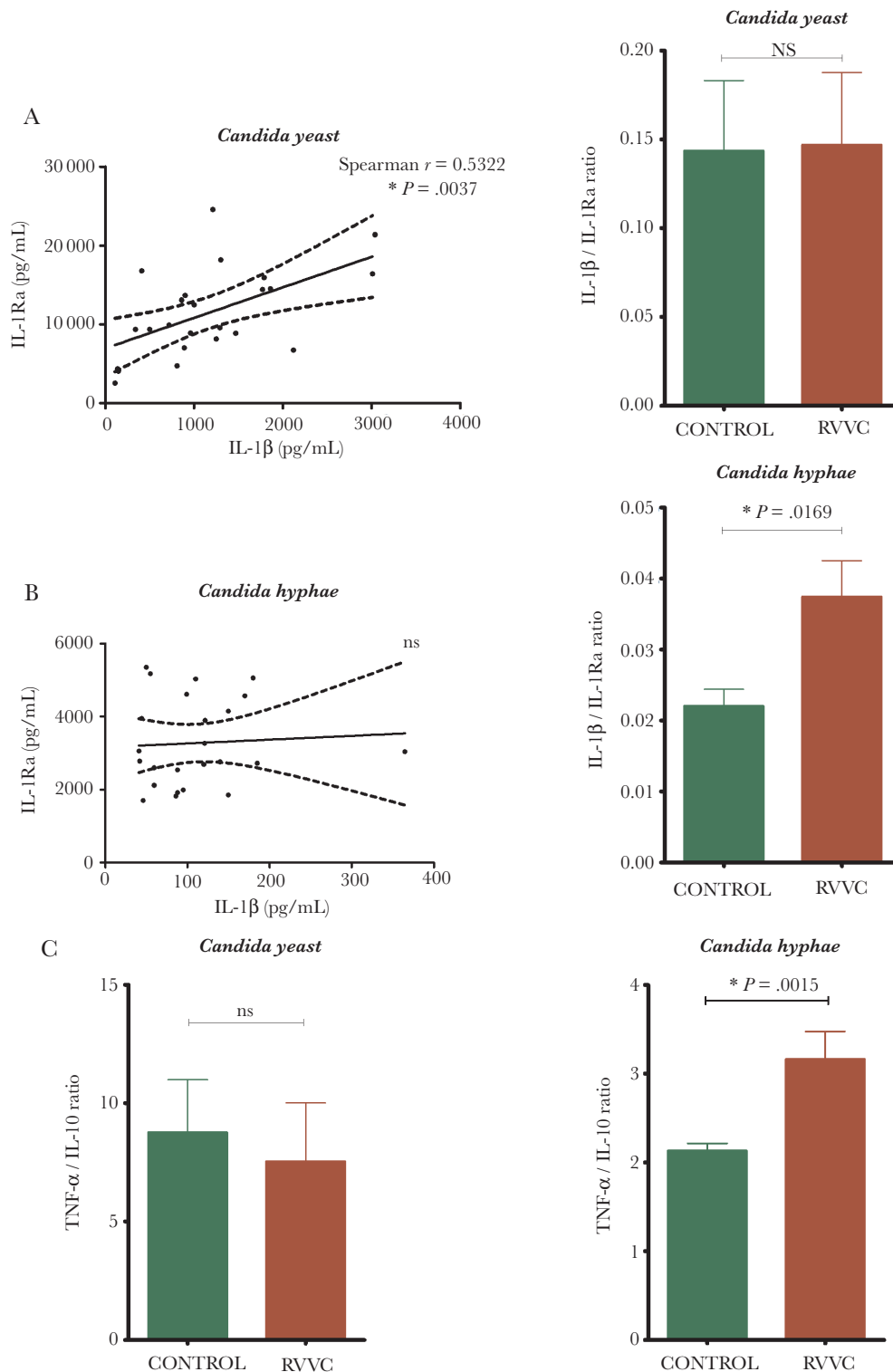


Figure 4. Increased IL-1 β bioactivity. *A*, Left, correlation between IL-1 β and IL-1Ra in PBMCs from RVVC patients stimulated with *Candida albicans* yeast HK (UC820, 1×10^5 cells/mL). Right, difference in ratio of IL-1 β and IL-1Ra levels between controls and patients. *B*, Left, correlation between IL-1 β and IL-1Ra in PBMCs from RVVC patients stimulated with *C. albicans* hyphae HK (UC820, 1×10^6 cells/mL). Right, difference in ratio of IL-1 β and IL-1Ra levels between controls and patients. *C*, Difference in ratio of IL-1 β and IL-1Ra levels between controls and patients in PBMCs exposed to *C. albicans* yeast (left) and hyphae (right). Correlations were analyzed using Spearman rank correlation coefficient test. $* P$ value $< .05$ was considered statistically significant. Abbreviations: HK, heat killed; IL-1Ra, interleukin-1 receptor α ; IL-1 β , interleukin-1 β ; NS, nonsignificant; PBMC, peripheral blood mononuclear cell; RVVC, recurrent vulvovaginal candidiasis.

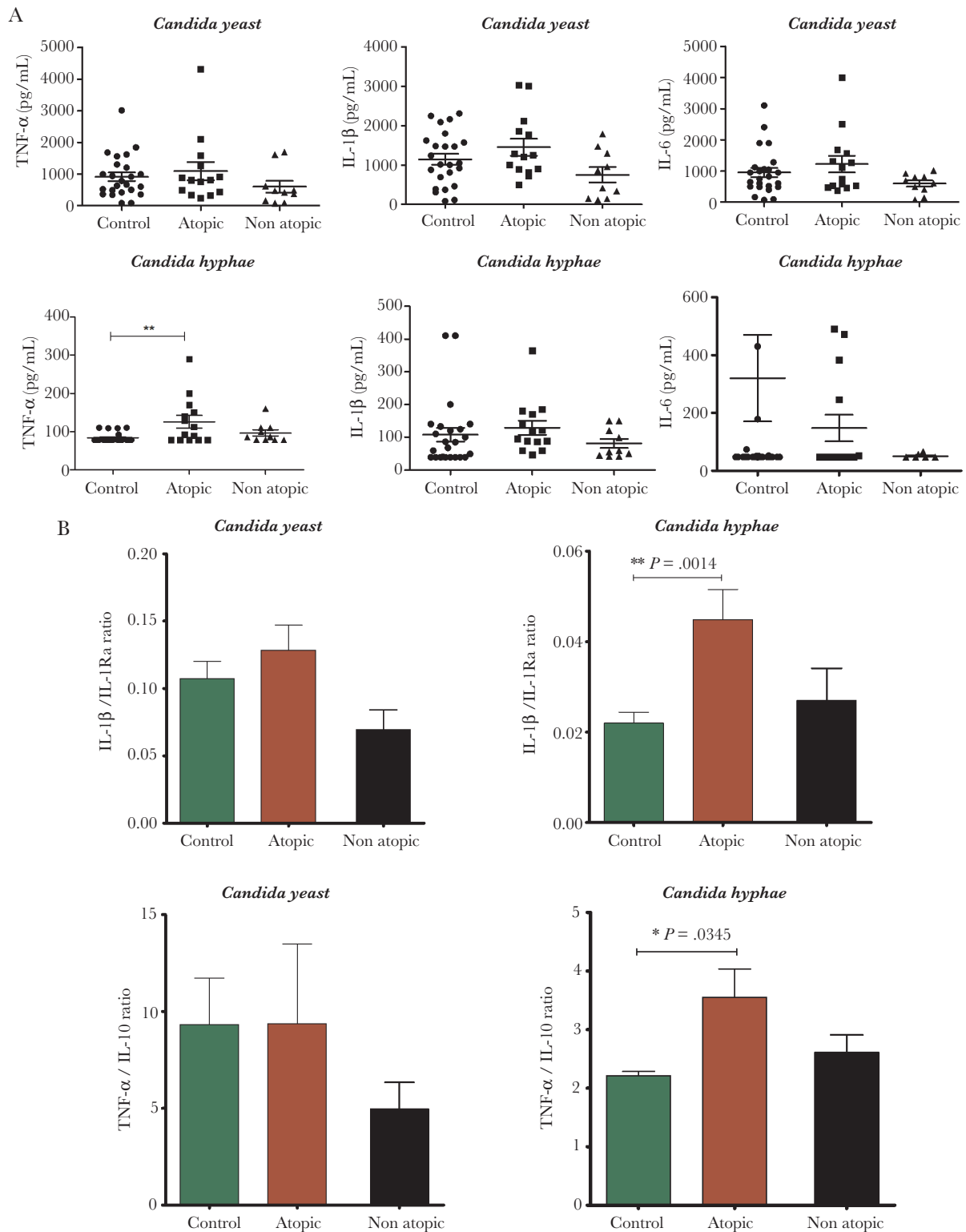


Figure 5. The influence of atopy on immune response. RVVC patients were divided into 2 clinical subgroups based on atopic constitution. A, Cytokine production capacity (TNF- α , IL-1 β , and IL-6) in the 2 subgroups and healthy controls in PBMCs culture supernatants after 24 hours stimulation with *Candida albicans* yeast (above) and hyphae (below). Results are mean \pm SEM. Significance was determined with Mann-Whitney *U* test. B, Above, difference in IL-1 β /IL-1Ra ratio between controls and patients in PBMCs exposed to *C. albicans* yeast (left) or hyphae (right). Below, difference in TNF- α /IL-10 ratio between controls and patients in PBMCs exposed to *C. albicans* yeast (left) or hyphae (right). Correlations were analyzed using Spearman rank correlation coefficient test. * *P* value < .05 was considered statistically significant. Abbreviations: IL, interleukin; IL-1Ra, interleukin-1 receptor α ; PBMC, peripheral blood mononuclear cell; RVVC, recurrent vulvovaginal candidiasis; SEM, standard error of the mean; TNF- α , tumor necrosis factor- α .

RVVC pathophysiology. In line with our results, prior studies revealed that RVVC does not occur in patients with genetic defects in adaptive immunity leading to mucosal fungal infections: for example, deficiencies of STAT3, a signal mediator activated also in response to type I [17] and type II [18] interferon, or mutations in IL-12/IL-12R signaling pathway, lead to more frequent oral mucosal candidiasis or different intracellular pathogen infections (ie, mycobacterial infections [19]) rather than to RVVC [20]. In contrast to Carvalho et al [21], we found only a slight decrease of IFN- γ in RVVC patients in response to *C. albicans* antigens and normal IL-10 concentrations between the 2 groups, suggestive for very little dysregulation of Th1/Th2-related immunity. In addition, we did not find diminished IL-17 production, similarly to previous findings reporting a normal systemic CMI [22, 23]. Interestingly, the decrease in absolute CD4⁺ T-lymphocyte count characteristic of HIV patients, inborn errors in the IL-17 pathway (eg, IL-17RA, IL-17F), or mutations in *STAT1*, *STAT3*, and *AIRE* [24, 25], resulting in chronic mucocutaneous candidiasis, hyper IgE syndrome, and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, are commonly associated with fungal infections such as cutaneous and oropharyngeal candidiasis [26] but not RVVC. The role of IL-22 in VVC is still not clear: while in vitro studies reported that the absence of IL-22 dampens IL-1Ra production, resulting in unrestrained inflammasome function and symptomatic VVC [27], we found normal IL-22 production capacity in RVVC patients. Moreover, individuals bearing a polymorphism in the novel candidate susceptibility factor SIGLEC15 have been reported recently to have high IL-22 concentrations as well as IL-17A and IFN- γ [28]. Taken together, these observations suggest that defects in IL-22 signaling pathway are not the primary cause of RVVC, at least not in most patients.

C. albicans is a dimorphic fungus and its switch from yeast to hyphae morphology mirrors the shift from a commensal to an opportunistic relationship with the host. The ability to form hyphae is required for the exuberant inflammatory response [29, 30], as supported by the induction of less immunopathology by *Candida* strains defective in the yeast to hyphae transition, like *C. glabrata* [29]. The lower rate of infection of *C. glabrata* and other non-*albicans* species, excluding *C. tropicalis*, as opposed to *C. albicans* (present in around 90% of cases) at the vaginal mucosa, is likely attributable to their inability to form hyphae. Nevertheless, *C. glabrata* is the second leading cause of VVC as well as severe candidiasis, meaning there are other morphology-independent virulence factors, such as candidalysin expression, biofilm formation, and phospholipase secretion, responsible for vaginitis immunopathology [30–32].

Lastly, we did not find any significant alterations in dectin-1 function overall in our cohort. However, 3 of 24 patients did not display a synergistic effect when PBMCs were costimulated with β -glucan and LPS: we cannot exclude therefore a partial dectin-1 defect in a minority of RVVC patients. It is

important to note that, in particular, a complete loss-of-function mutation of dectin-1 is associated with increased susceptibility to mucocutaneous *Candida* infection [33, 34]. Homozygous individuals dectin-1 Tyr238X deficient are rare in the western population [35], explaining why dectin-1 deficiency is a risk factor at the individual level but it is not responsible for RVVC susceptibility in the overall RVVC population [36]. Presumably, due to the small number of patients in our cohort and the rarity of the loss-of-function phenotype in the population, we did not include any homozygous individuals. This suggests that other individuals with the clinical phenotype are most likely characterized by an autoinflammatory condition rather than a genetic deficiency.

Interestingly, in our cohort more than half of the patients recruited for this study were diagnosed with atopic disease and increased IL-1 β and TNF- α bioactivity were measured in comparison to healthy individuals. These results further strengthen the hypothesis that RVVC is a hypersensitivity response and that atopy might play a role in the pathogenesis of RVVC rather than being a confounder, as also suggested in other studies [14, 37]. The biological mechanisms that link atopy and RVVC, however, still need to be elucidated.

The main limitation of this study is that we measured cytokine production in supernatant from PBMCs and not in vaginal fluids. Further studies are warranted to investigate the crucial role of the microbiome, metabolome, and inflammatory mediators (ie, PMNs) in vaginal anti-*Candida* immunity.

In conclusion, we provide evidence that women with RVVC are characterized by an inappropriately strong cytokine production profile that is reminiscent of an autoinflammatory disease, rather than a defect in T-cell mediated immunity. A hallmark is the increased bioactivity of IL-1 β in response to invasive *C. albicans* infection, which is inadequately counterbalanced by IL-1Ra production, thus, resulting in recurrent *Candida* vaginitis. This potentially offers the opportunity for new treatment options aimed at reducing the excessive inflammation.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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