INFECTION AND IMMUNITY, May 2000, p. 2464–2469 Vol. 68, No. 5
0019-9567/00/$04.00+0

Candida albicans and *Candida krusei* Differentially Induce Human Blood Mononuclear Cell Interleukin-12 and Gamma Interferon Production

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Received 22 November 1999/Returned for modification 22 December 1999/Accepted 26 January 2000

Protection against *Candida* infection involves both innate and acquired immune responses, and cytokines produced by monocytes during the innate response may modify the acquired immune response by T cells. We hypothesized that *Candida* species which differ in pathogenicity can differentially induce production of immunoregulatory cytokines by human monocytes, which in turn modify T cells for immune responses to *Candida*. To test this hypothesis, we examined the effects of *Candida albicans* and *Candida krusei* on immunoregulatory cytokine production by human monocytes and gamma interferon (IFN-γ) production by peripheral blood mononuclear cells (PBMC). Purified monocytes were incubated with live or heat-killed strains of *C. albicans* and *C. krusei* at the optimal *Candida*/monocyte ratio of 0.5. Cytokines in the supernatants were measured by enzyme-linked immunosorbent assay. Our data demonstrated that live *C. albicans* and *C. krusei* significantly induced interleukin-10 (IL-10), monocyte chemotactic factor 1, IL-1β, and tumor necrosis factor alpha production by monocytes relative to unstimulated monocytes. In contrast, unlike *C. krusei*, pathogenic live strains of *C. albicans* induced no or only a minimal level of IL-12. The expression of IL-12 p40 mRNA levels by reverse transcription-PCR corroborated the IL-12 protein (p70) findings. In human PBMC, human blood monocytes were the major source of both IL-10 and IL-12 production in response to *C. albicans* and *C. krusei*. Upon activation of T cells in the presence of *Candida*-modified monocytes and antigen-presenting cells, IL-12 production by PBMC treated with *Candida* organisms correlated strongly with the level of IFN-γ production by T cells. These results indicate that the virulence of *C. albicans* may be related to its ability to induce the monocytic type II cytokine IL-10, with a selective inhibition of IL-12 production, which may be responsible for the observed lack of T-cell IFN-γ and may restrain an effective type I immune response to *Candida*.

Candida albicans is a major opportunistic fungal pathogen which may be present in humans as a commensal microbial flora; most importantly, it causes candidiasis in immunocompromised hosts due to malignant tumors, major surgery, organ transplantation, or treatment with cytotoxic or immunosuppressive drugs (13). In addition to *C. albicans*, other *Candida* species, even much less virulent non-*albicans Candida* species such as *C. krusei* (1, 13, 36, 42, 43), have been reported as pathogens causing systemic candidiasis.

The importance of polymorphonuclear leukocytes has been extensively studied in the pathogenesis of candidiasis (4, 5, 33). However, systemic candidiasis has also occurred in hosts with normal neutrophil function, suggesting that cells other than neutrophils also play an important role in host defense. When hosts are neutropenic, mononuclear cells, especially monocytes/macrophages, contribute to the defense against the infection (17); nonetheless, their functions in pathogenesis of candidiasis in humans have not been fully explored.

Monocytes have the capacity to produce chemokines (23), proinflammatory cytokines (6), and particularly the immunoregulatory cytokines interleukin-10 (IL-10) and IL-12 (9, 41). Immunoregulatory cytokines released as a result of the initial contact of *Candida* with host monocytes/macrophages can also be a major factor, which can potentially regulate the acquired immune response through T-cell development, in host defense. IL-12 is essential for inducing type I immune responses, and the development of gamma interferon (IFN-γ)-producing T cells (25, 41), which in turn are associated with resistance to candidal infection (34, 35). Its reciprocal immunoregulatory cytokine, IL-10, inhibits IL-12 and IFN-γ production (2, 8, 24), favoring type II immune responses (8, 9, 11), which are associated with susceptibility to *C. albicans* infection (7, 40).

We hypothesized that *Candida* species which differ in pathogenicity can differentially induce production of immunoregulatory cytokines by human monocytes, which in turn modify T cells for immune responses to *Candida*. In this study, we show that *C. albicans* and *C. krusei* differentially induce IL-12 production; i.e., *C. krusei*, but not *C. albicans*, clearly induced IL-12 by monocytes. These results indicate that pathogenic *Candida* species have the ability to create an environment rich in IL-10 and poor in IL-12 and IFN-γ, which would generate a more susceptible state of the host to candidiasis.

MATERIALS AND METHODS

PBMC, monocytes, and nonadherent mononuclear cells (NAC). Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood of healthy volunteer by Histopaque-1077 (Sigma Chemical Company, St. Louis, Mo.) gradient centrifugation. Monocytes were isolated by incubation of PBMC in tissue culture dishes for 1 h at 37°C followed by harvesting adherent cells using 0.5 mM EDTA in Hanks balanced salt solution (Life Technologies, Grand Island, N.Y.). The T, B, and natural killer cells and erythrocytes following treatment with antibody mixture (anti-CD2, -3, -19, -56 and -glycophorin A) and dextran-iron (as instructed by the manufacturer [Stem Cell Technologies, Vancouver, British Columbia, Canada]) were removed by adherence to a MACS

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separation column against a MidiMACS magnet (Miltenyi Biotec, Auburn, Calif.) (44).

NAC were collected after PBMC adherence to plastic dishes at 37°C for 1 h in a 5% CO_2 incubator. The cells were adjusted to 2×10^6/ml in RPMI 1640 plus penicillin, streptomycin, and 10% fetal bovine serum. Endotoxin was determined in the supernatants by using E-Toxate reagent (Sigma).

Fungal organisms. The *C. albicans* strains (SC5314, 1442, 2307, and 2183) and *C. krusei* isolates (6258 and A-L) used in this study have been described previously (15, 16, 21). *Candida* strains were stored in a mixed medium composed of glycerol and Sabouraud's dextrose broth (1:1) at −70°C. The organisms were streaked onto Sabouraud's dextrose agar, and the plates were incubated at 37°C overnight. One colony was transferred to 10 ml of Sabouraud's dextrose broth, and the cells were incubated overnight at 37°C in a shaking water bath. The organisms were centrifuged at 1,500 rpm for 8 min, washed twice with phosphate-buffered saline (PBS), then resuspended in RPMI 1640 plus penicillin, streptomycin, and 10% FBS, and adjusted to a final concentration of 10^7 cells/ml. In some experiments, organisms were heat killed by suspension in RPMI 1640 by incubation in a 60°C water bath for 30 min.

Coculture of fungal cells with PBMC, monocytes, and NAC. To prevent stimulation of blood cells with plastic, six-well plates were coated with 0.05% bovine serum albumin in PBS for 1 h, followed by three washes with PBS. PBMC, monocytes, or NAC (1 ml of 2×10^6 cells/ml) were added to each well and incubated for 45 min. Then yeast cells were added to the blood cells at different ratios (see Results) and incubated for 20 h at 37°C in a 5% CO_2 incubator. Following incubation, supernatants were collected and stored at −70°C until use. The viability of monocytes in coculture with live *C. albicans* and *C. krusei* was monitored using a lactate dehydrogenase (LDH) assay kit (Boehringer Mannheim Corporation, Indianapolis, Ind.).

Cytokine ELISA. Cytokine proteins in cell supernatants were quantitated by enzyme-linked immunosorbent assay (ELISA) with antibody pairs for either IL-12, tumor necrosis factor alpha (TNF-α; R&D Systems Inc., Minneapolis, Minn.), IL-1β (Endogen, Woburn, Mass.), IFN-γ, monocyte chemotactic protein 1 (MCP-1), IL-4, or IL-10 (PharMingen International, San Diego, Calif.). The sensitivity of all ELISAs was ≥10 pg/ml.

RNA extraction and RT-PCR. Reverse transcription-PCR (RT-PCR) was performed as previously described (19). Briefly, total RNA of monocytes was extracted by an RNeasy Total RNA kit (Qiagen, Chatsworth, Calif.) and quantified by spectrophotometric measurement. cDNA was synthesized from 200 ng of total RNA. The primers used for PCR were as follows: IL-12 p40 (nucleotides 806 to 822 of sense strand [5′-CCACATTCCTACTTCTC-3′] and nucleotides 1061 to 1077 of antisense strand [5′-GTCTATTCCGTTGTGTC-3′]; 272 bp) and β-actin (447 bp). Thirty-two cycles were conducted in Quarther Bath Thermal Cycler (Inotech, Lansing, Mich.) with denaturation at 94°C for 1 min, annealing at 55°C (60°C for β-actin) for 1 min, and 72°C for 2 min. PCR products were electrophoresed with 2% agarose gel with ethidium bromide.

Statistics. Results were expressed as mean ± standard error for n number of repeat experiments. Statistical significance was determined by Student's t test. The correlation coefficient of two variables was evaluated by using linear regression, and statistical significance was determined by t test. A P value of <0.05 was considered significant.

RESULTS

***C. albicans* as well as *C. krusei* induced MCP-1, IL-1β, and TNF-α by human blood monocytes.** To determine whether live *C. albicans* and *C. krusei* can differentially induce cytokine production by human blood monocytes, we first tried to determine the optimal ratio of *Candida* organisms to monocytes for use in subsequent experiments. The yeast/monocyte ratios examined were 0.001, 0.01, 0.05, 0.1, 0.5, 1, 10, and 50 (data not shown). These preliminary experiments showed that a yeast/monocyte ratio of 0.5 caused significant stimulation of IL-1β and TNF-α production by monocytes. A lower yeast/monocyte ratio (0.01) was needed to cause significant induction of MCP-1. At the ratio of 0.5, *Candida* organisms caused no statistically significant increase of LDH release by monocytes relative to unstimulated controls. Therefore, the ratio of 0.5 was chosen to determine the immunoregulatory cytokine production by monocytes following stimulation by *Candida* cells in all subsequent experiments. As shown in Table 1, both *C. albicans* SC5314 and *C. krusei* 6258 induced high and significant levels of MCP-1, IL-1β, and TNF-α production by monocytes relative to unstimulated controls ($P < 0.05$). The endotoxin level in the supernatants was determined and found to be below the detectable level (<0.06 endotoxin unit/ml), indicating that our preparations were not contaminated by endotoxin.

TABLE 1. Live *C. albicans*- and *C. krusei*-induced IL-1β, TNF-α, and MCP-1 production by monocytes[a]

Monocyte treatment	Mean concn (pg/ml) ± SD (n)		
	IL-1β	TNF-α	MCP-1
Control	<10 (15)	<10 (15)	2,011 ± 619 (16)
C. albicans			
SC5314	1,823 ± 241* (11)	5,736 ± 329* (11)	7,561 ± 449* (16)
C. krusei 6258	1,885 ± 298* (5)	6,546 ± 617* (5)	8,581 ± 967* (16)

[a] Human peripheral blood monocytes were treated with live *C. albicans* SC5314 or *C. krusei* 6258. Supernatants were collected after incubation for 20 h. Cytokines were measured by ELISA. *, $P < 0.05$ versus control.

Unlike *C. krusei*, which significantly induced the production of IL-10 and IL-12 by human monocytes, *C. albicans* induced IL-10 production only. Although monocytes have the capacity to produce IL-10 and IL-12 (9, 20, 41), the ability of *C. albicans* to induce IL-10 and IL-12 by monocytes has not been investigated. Thus, in this study we examined the production of these two cytokines by monocytes following stimulation with *C. albicans* and *C. krusei*. Our results showed that both live *C. krusei* 6258 and live *C. albicans* SC5314 stimulated IL-10 production; however, *C. krusei* 6258 but not *C. albicans* SC5314 induced significant IL-12 production by monocytes (32 ± 12 pg/ml for *C. krusei* versus 0 pg/ml for both *C. albicans* and controls; $P < 0.05$) (Fig. 1). These results indicate that production of the immunoregulatory cytokine IL-12 by monocytes is differentially induced by different *Candida* species and differs from its reciprocal counterpart IL-10.

To determine whether induction of IL-12 production by monocytes with *C. krusei* but not *C. albicans* is species or strain specific, we repeated the above experiments using additional strains of these candidal species. Three *C. albicans* strains (1442, 2307, and 2183) have been previously characterized for

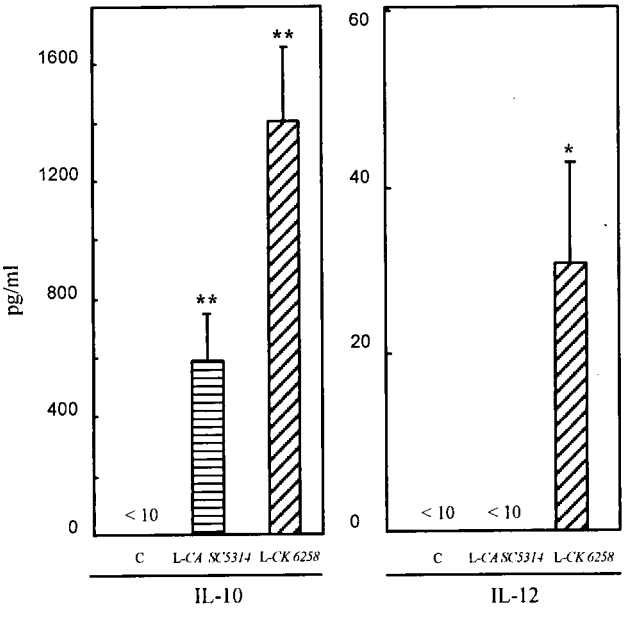

FIG. 1. IL-10 and IL-12 production by monocytes treated with live *C. albicans* SC5341 or live *C. krusei* 6258. Human PBMC were treated with live *C. albicans* SC5314 (L-*CA SC5314*) or *C. krusei* 6258 (L-*CK 6258*). Supernatants were collected after incubation for 20 h. Cytokines were measured by ELISA. Monocytes without fungal cells were used as controls (C). $n = 18$ for IL-10; $n = 16$ for IL-12; *, $P < 0.05$ versus control; **, P, <0.01 versus control.

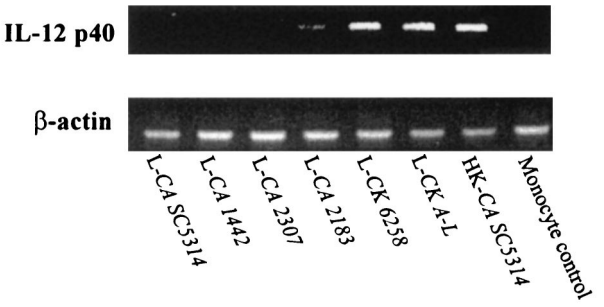

FIG. 2. Expression of IL-12 p40 mRNA in human peripheral blood monocytes was inhibited by *C. albicans* species. Human peripheral blood monocytes were cocultured with live *C. albicans* SC5314, 1442, 2307, and 2183 (L-*CA SC5314*, L-*CA 1442*, L-*CA 2183*, and L-*CA 2307*, respectively), *C. krusei* 6258 and A-L (L-*CK 6258* and L-*CK A-L*, respectively), or monocytes alone as control for 20 h. Total RNA was extracted, and RT-PCR was conducted with primers for IL-12 p40 and β-actin. Results are representative of two separate experiments that yielded similar results.

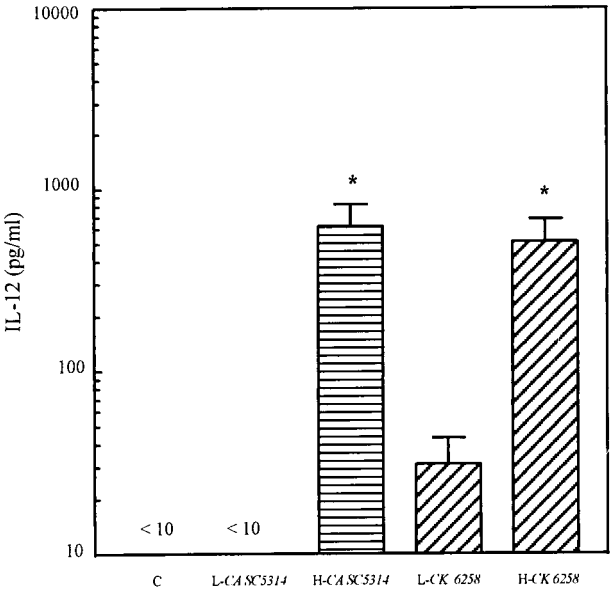

FIG. 3. IL-12 production by monocytes treated with live *C. albicans* SC5314, live *C. krusei* 6258, heat-killed *C. albicans* SC5314, and heat-killed *C. krusei* 6258. Human peripheral blood monocytes were treated with live *C. albicans* SC5314 (L-*CA SC5314*), live *C. krusei* 6258 (L-*CK 6258*), heat-killed *C. albicans* SC5314 (H-*CA SC5314*), and heat-killed *C. krusei* 6258 (H-*CK 6258*). Supernatants were collected after incubation for 20 h. Cytokine was measured by ELISA. Monocytes without fungal cells were used as control (C). $n = 16$ for L-*CA* and L-*CK*; $n = 6$ for H-*CA SC5314* and H-*CK 6258*; *, $P < 0.05$ versus L-*CA SC5314* or L-*CK 6258*.

their virulence (16). The first strain is highly virulent, while the other two are of low virulence as determined by animal survival studies (16). A second *C. krusei* strain (A-L) was also included in these experiments. In general, *C. albicans*, unlike *C. krusei*, failed to induce IL-12 production by monocytes (levels of <10 pg/ml). However, one strain of *C. albicans* that was characterized by Graybill's group (16) to be of low virulence (strain 2183) stimulated the production of minimal but insignificant ($P > 0.05$) amount of IL-12 p70 protein (10 ± 5 pg/ml) by monocytes. *C. krusei* 6258 and A-L stimulated IL-12 production to levels of 31 ± 12 ($n = 16$) and 283 ± 167 pg/ml ($n = 4$).

Next we performed RT-PCR to determine whether IL-12 p70 protein production is mirrored at the mRNA level. As shown in Fig. 2, the pattern of IL-12 p40 mRNA expression paralleled IL-12 p70 protein production following stimulation with *C. krusei* (strains 6258 and A-L both caused remarkable expression of IL-12 p40 mRNA). In contrast, *C. albicans* did not stimulate or induced a minimal level (strain 2183) of mRNA expression (Fig. 2). As expected, monocytes incubated in the absence of fungal cells as a negative control did not express IL-12 p40 mRNA (Fig. 2).

IL-12 is markedly induced by human blood monocytes treated with heat-killed *C. albicans* and *C. krusei*. To determine whether viability is critical for the inability of *C. albicans* to induce IL-12, *C. albicans* SC5314 and *C. krusei* 6258 were heat killed and cocultured with monocytes. IL-12 production by monocytes treated with heat-killed and live *C. albicans* SC5314 or *C. krusei* 6258 was monitored. As expected, live *C. albicans* failed to induce IL-12 whereas live *C. krusei* did. In contrast, heat-killed *C. albicans* and *C. krusei* induced high levels of IL-12 production by monocytes ($P < 0.05$) (Fig. 3), indicating that the inhibition of IL-12 production by *C. albicans* is an active process requiring viable *Candida* cells.

Human blood monocytes represent the major source of IL-10 and IL-12 production by PBMC in response to *C. albicans* and *C. krusei* stimulation. Although purified monocytes in the above experiments were clearly capable of responding to *Candida* directly, it is possible that in vivo, where complex mixtures of immunocytes interact, other cell types might participate in or regulate monocyte immunoregulatory cytokine production. To determine if monocytes are the major producer of IL-10 in the mix of T cells, B cells, monocytes, dendritic cells, basophils and NK cells contained in PBMC, whole PBMC were cocultured with either live *C. albicans* SC5314 or live *C. krusei* 6258. IL-10 protein in the supernatants was determined and compared between purified monocytes and PBMC treated with the same candidal strain. Monocytes/PBMC at the concentration of 2×10^6/ml with the yeast-to-monocyte/PBMC ratio of 0.5 were cultured as described above. The percentage of monocytes in the purified monocyte preparations was >90%, whereas in PBMC monocytes represented only 7.4% ± 2.1% ($n = 2$; as determined by flow cytometry for CD14); this represented an approximately 12-fold enrichment. IL-10 was significantly higher in the supernatants of cultured monocytes stimulated with either live *C. albicans* SC5314 ($P < 0.05$) or *C. krusei* 6258 ($P < 0.01$) relative to that in the PBMC supernatants (Fig. 4). Next, to determine if the monocytes are the major producer of IL-12 in stimulated PBMC, monocytes, PBMC, and NAC were cocultured with live *C. krusei* 6258, and IL-12 protein levels in the supernatants were determined. Since live *C. albicans* SC5314 was unable to stimulate monocytes and PBMC to produce IL-12, it was not included in these experiments. After adherence, the monocytes, as determined by flow cytometry, in NAC dropped to 2.7% ± 0.8%, compared to 7.4% ± 2.1% in PBMC. Our data show that IL-12 was not detected in NAC cocultured with *C. krusei*, while live *C. krusei* induced significantly higher IL-12 production by PBMC (80 ± 32 pg/ml; $n = 6$ [Table 2]) than that of NAC ($P < 0.05$). Because different cell types within PBMC regulate IL-12, we were unable to show significance in the production of this cytokine between PBMC and enriched monocytes. Taken together, these results indicate that monocytes are the major producer of both IL-10 and IL-12 in PBMC after *Candida* stimulation.

IFN-γ induction correlates strongly with the level of IL-12 in the supernatants of PBMC treated with *Candida* organisms. Production of IFN-γ by T cells is dependent on IL-12 and is

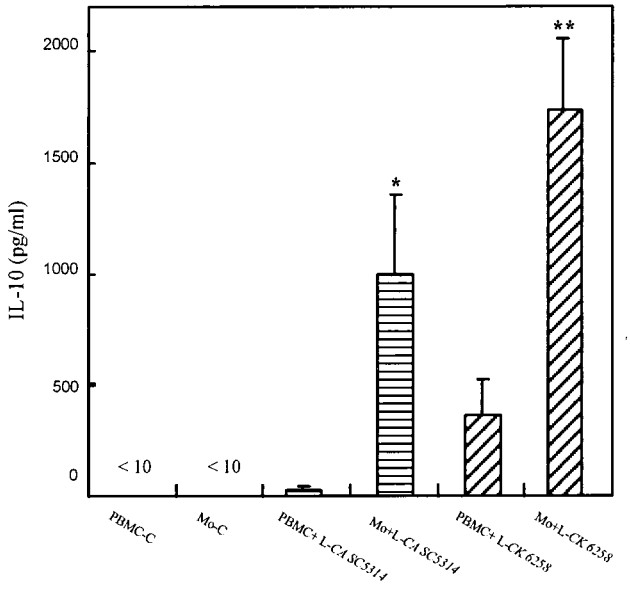

FIG. 4. Comparison of IL-10 production by human blood monocytes treated with live *C. albicans* SC5314 and *C. krusei* 6258 to that by PBMC. Monocytes (Mo) and PBMC were treated with live *C. albicans* SC5314 (L-*CA SC5314*) or *C. krusei* 6258 (L-*CK 6258*). Supernatants were collected after incubation for 20 h. Cytokine was measured by ELISA. Monocytes and PBMC without fungal cells were used as controls. PBMC-C, PBMC control; Mo-C, monocyte control; $n = 6$; *, $P < 0.05$ versus PBMC+L-*CA SC5314*; **, $P < 0.01$ versus PBMC+L-*CK 6258*.

critical for immune responses associated with cell-mediated immunity against microorganisms, including *Candida*. To determine if a correlation exists between the inability of *C. albicans* to induce IL-12 and IFN-γ production, PBMC were incubated with live or heat-killed *C. albicans* SC5314 or *C. krusei* 6258, and the supernatants were assayed for the simultaneous presence of IL-12 and IFN-γ. As can be seen in Table 2, live *C. albicans* SC5314 failed to produce either IL-12 or IFN-γ. In contrast, stimulation of PBMC with either *C. krusei* 6258 or heat-killed *C. albicans* led to the production of significant amounts of both IL-12 and IFN-γ. IL-4 was not detectable in the supernatants of PBMC treated with live *C. albicans* SC5314 and *C. krusei* 6258 (data not shown). Therefore, these data indicated that IFN-γ production by PBMC strongly correlated with IL-12 production ($r^2 = 0.81$, $P < 0.01$ for heat-killed *C. albicans* SC5314; $r^2 = 0.84$, $P < 0.01$ for live *C. krusei* 6258; $r^2 = 0.82$, $P < 0.01$ for heat-killed *C. krusei* 6258) (Table 2).

TABLE 2. Correlation of IL-12 and IFN-γ production by PBMC treated with *Candida* organisms[a]

Treatment	Mean concn (pg/ml) ± SD ($n = 6$)		r^2	P
	IL-12	IFN-γ		
C. albicans SC5314				
Live	<10	<10		
Heat killed	639 ± 158	1,281 ± 586	0.81	<0.01
C. krusei 6258				
Live	80 ± 32	459 ± 620	0.84	<0.01
Heat killed	440 ± 106	1,201 ± 570	0.82	<0.01

[a] PBMC were treated with live or heat-killed *C. albicans* SC5314 and *C. krusei* 6258. Supernatants were collected after incubation for 20 h. Cytokines were measured by ELISA.

DISCUSSION

In this study, we showed that *C. albicans*-stimulated monocytes produced high levels of MCP-1 and proinflammatory cytokines IL-1β and TNF-α (Table 1). These findings agree with earlier reports (3, 6, 10, 18). Production of these cytokines under the influence of *C. albicans* may enhance acute inflammatory cell influx into infected tissues, activate leukocytes, and promote early noncognate elimination of the organism prior to the development and/or mobilization of an adaptive specific immune response (30, 38). Furthermore, we found that clinical isolates of *C. albicans* and *C. krusei* which differ in pathogenicity can also induce similar levels of the immunoregulatory cytokine IL-10. Interestingly, these yeast species differentially induce IL-12 production by monocytes.

It has been well documented that IL-10 plays an inhibitory role in monocytes and neutrophils against *Candida* (7, 29, 39). In the murine models of candidiasis, neutralization of IL-10 upregulates nitric oxide production and protects susceptible mice from challenge with *C. albicans* (28, 33). This indicates that IL-10 suppresses protective type I responses in mice with *C. albicans* infection. The ultimate net response in vivo may depend on host immunogenetics and immune status and the virulence of the fungal strain.

Our data showed that live *C. albicans* SC5314 failed to induce IL-12 production whereas *C. krusei* 6258 induced the production of significant levels of IL-12 by monocytes ($P < 0.05$). To determine whether this observation is strain specific or species specific, we extended our studies to include three additional strains of *C. albicans* and one strain of *C. krusei*. The relative virulence of the three strains of *C. albicans* was shown to be 1442 > 2307 > 2183 (16). Similar to *C. albicans* SC5314, the virulent strain 1442 failed to induce IL-12 production by monocytes. Although *C. albicans* 2307 and 2183 (low-virulence strains) induced a trace amount of IL-12, this level was not statistically significant. In contrast, A-L, the second *C. krusei* strain tested, like *C. krusei* 6258, induced high levels of IL-12 production by monocytes (see Results).

Failure to detect IL-12 in the culture of monocytes treated with *C. albicans* was also shown by mRNA level. The expression of IL-12 p40 mRNA by monocytes stimulated with *C. albicans* and *C. krusei* was in line with the IL-12 p70 protein levels (Fig. 2). Thus, the possibility of extrinsic factors such as absorption or degradation by *C. albicans* in the medium which might affect detection of IL-12 is excluded.

The mechanism(s) responsible for the inhibition of IL-12 induction by monocytes needs to be explored. We asked whether *C. albicans* essentially failed to produce IL-12 by monocytes is due to the viability of *C. albicans*. Our data showed that both heat-killed *C. albicans* and heat-killed *C. krusei* induce high levels of IL-12 production by monocytes (Fig. 3). Overall, it is quite clear that live virulent and avirulent strains of *C. albicans* are all substantially incapable of stimulating IL-12 production, the true distinction being that between live and inactivated cells. These results indicate that inhibition of IL-12 production by monocytes is an active process on the part of *C. albicans* and may be associated with virulence factors such as germination, production of enzymes (such as phospholipase), and complement activation. It is likely that phagocytosis of yeasts by monocytes is involved in the production of IL-12, because *C. krusei* does not make true, uningestible hyphae and heat-killed *C. albicans* does not make hyphae either. Fulton et al. (14) reported that inhibition of phagocytosis by cytochalasin D reduced the IL-12 p40 mRNA expression by monocytes treated with *Mycobacterium tuberculosis. C. albicans* appears as both yeast and hyphae in RPMI 1640; therefore,

whether hyphae and phagocytosis of yeasts play a role in regulation of IL-12 production by monocytes is currently being investigated.

We further determined whether monocytes are the main source of the immunoregulatory cytokines IL-10 and IL-12 in PBMC treated with *Candida* species. Levitz and North (22) reported that PBMC treated with heat-killed *C. albicans* could produce IL-10 and postulated that IL-10 could be dependent on the presence of peripheral blood monocytes. We compared the IL-10 production by monocytes and PBMC treated with both live *C. albicans* and live *C. krusei*. We also compared IL-12 protein production by PBMC and NAC treated with live *C. krusei*, in which monocytes were depleted by adherence. As shown in Results, monocytes are the main source of IL-10 and IL-12 in PBMC treated with these *Candida* organisms. The importance of monocytes as the main source of IL-10 and IL-12 in PBMC is that they exert a central functional effect on differentiation of immune responses. To determine whether the production of IL-12 by monocytes stimulated with *Candida* organisms affected pivotal T-cell function, IFN-γ was monitored along with IL-12 in the supernatants of PBMC cocultured with the *Candida* organism. Our results show that IFN-γ production was absent upon stimulation with *C. albicans* but clearly induced by the low-virulence *C. krusei* and heat-killed *C. albicans*. IFN-γ production correlated strongly with IL-12 production in PBMC challenged with *Candida* species (Table 2). It is well documented that a type I immune response is characterized by increased IFN-γ, which can enhance the antifungal activity of neutrophils (31, 32, 37) in vitro and can protect endothelial cells from organism-induced damage (12, 35), thereby leading to host resistance and onset of protective immunity (27, 28, 38). In contrast, IL-10 inhibits type I cell development and favors susceptibility to *C. albicans* (26, 33). Therefore, the ability of live *C. albicans* to induce IL-10 while failing to induce IL-12 production by monocytes may create a circumstance in which type I response is suppressed, thus increasing the susceptibility of the host to candidiasis. In the case of *C. krusei*, however, IL-12 was clearly induced by monocytes, which leads to type I response and favors cell-mediated immunity against *C. krusei* infection. These results may partly explain the high and low incidences of infections due to *C. albicans* and *C. krusei*, respectively. IFN-γ was induced, while IL-4 was not detectable, in PBMC treated with live *C. krusei*, indicating that a type II immune response was not induced in place of a type I response, at least upon a single round of stimulation. Taken together, these results indicate that the virulence of *C. albicans* may be related to its ability to selectively induce IL-10, with simultaneous inhibition of monocytic IL-12 and T-cell IFN-γ.

ACKNOWLEDGMENTS

This work was supported in part by grants from Pfizer Pharmaceutical Group, New York (M.A.G. and K.K.), National Institutes of Health grants AI-35097-04 (M.A.G.) and AI-41766 (K.D.C.), and the University Hospitals of Cleveland Research and Education Fund.

We thank John R. Graybill (University of Texas San Antonio) for providing *C. albicans* virulence strains 1442, 2307, and 2183, Steven D. Leidich and Chad Jessup for preparing the other *Candida* isolates, and Guofen Chen for technical assistance.

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Editor: T. R. Kozel