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Candida albicans Stimulates IL-23 Release by Human Dendritic Cells and Downstream IL-17 Secretion by Vδ1 T Cells

Christina O. Maher,* Katie Dunne,† Ross Comerford,‡ Siobhán O’Dea,‡ Aisling Loy,‡
James Woo,‡ Thomas R. Rogers,‡ Fiona Mulcahy,‡ Pádraic J. Dunne,*1 and
Derek G. Doherty,**1

γδ T cells expressing the Vδ1 TCR are expanded in patients with HIV infection. We show in this article that circulating Vδ1 T cell numbers are particularly high in patients with HIV and candidiasis, and that these cells expand and produce IL-17 in response to Candida albicans in vitro. Although C. albicans could directly stimulate IL-17 production by a subset of Vδ1 T cells, fungus-treated dendritic cells (DCs) were required to expand C. albicans–responsive Vδ1 T cells to generate sufficient numbers of cells to release IL-17 at levels detectable by ELISA. C. albicans induced the release of IL-1β, IL-6, and IL-23 by DCs, but addition of these cytokines or supernatants of C. albicans–treated DCs to Vδ1 T cells was not sufficient to induce proliferation. We found that direct contact with DCs was required for Vδ1 T cell proliferation, whereas IL-23R–blocking studies showed that IL-23 was required for optimal C. albicans–induced IL-17 production. Because IL-17 affords protection against both HIV and C. albicans, and because Vδ1 T cells are not depleted by HIV, these cells are likely to be an important source of IL-17 in HIV-infected patients with candidiasis, in whom CD4+ Th17 responses are impaired. These data show that C. albicans stimulates proliferation and IL-17 production by Vδ1 T cells by a mechanism that involves IL-17 release by DCs. The Journal of Immunology, 2015, 194: 5953–5960.

Candida albicans is a commensal yeast fungus that colonizes the oropharynx, intestine, and vagina in healthy individuals (1, 2). In HIV-infected individuals and other immunocompromised hosts, C. albicans can be a major pathogen, causing oropharyngeal, esophageal, or vulvovaginal candidiasis (3), and it is the fourth most common cause of nosocomial bloodstream infections in the United States with a 38% mortality rate (4). Identification of the mechanisms by which the immune system protects against C. albicans infection is critical for the development of treatments and vaccines against this pathogen.

Host defense against C. albicans requires the IL-17 pathway of cells and cytokines (5–8). IL-17 is an inflammatory cytokine released by a subset of CD4+ T cells (Th17 cells), some CD8+ T cells, NK cells, NK T cells, and innate lymphoid cells (9–11). IL-17 induces the production of antimicrobial peptides and chemokines, which recruit neutrophils to sites of infection where they kill extracellular bacteria and fungi by phagocytosis or the release of antimicrobial agents (6, 12, 13). Pathological C. albicans infections are common in humans with inherited deficiencies of the IL-17 pathway (such as IL-17 deficiency or mutations in the IL-17R or STAT1) (14, 15) and in knockout mice that lack IL-17 or IL-23 (6, 16). C. albicans–specific T cells in healthy donors are preferentially Th17 cells that coproduce IFN-γ (7).

T cells expressing γδ TCR chains are an important source of innate IL-17. Fenoglio and coworkers (17) showed that the Vδ1 subset of human γδ T cells proliferates and produces IFN-γ and IL-17 in response to C. albicans. However, the mechanisms by which C. albicans stimulates Vδ1 T cells are poorly understood. Murine γδ T cells expressing TLR2 and dectin-1, which bind β-glucans present in cells walls of fungi, are implicated in responses to C. albicans (8, 18–20). Murine γδ T cells can also release IL-17A and the Th17-associated cytokines, IL-17F, IL-21, and IL-22, in response to treatment with IL-1 and IL-23, or IL-18 and IL-23 in the absence of TCR stimulation (21, 22). Cellular sources of IL-1, IL-18, and IL-23 include monocytes, macrophages, dendritic cells (DCs), or neutrophils, which can express receptors that mediate immune recognition of C. albicans, such as TLR2, dectin-1, dectin-2, and mannose receptors (23, 24). IL-1β, IL-6, and IL-23 contribute to CD4+ Th17 cell induction by C. albicans in humans (7). However, the factors that induce and regulate IL-17 production by human Vδ1 T cells are not known.

Vδ1 T cells are expanded in the circulation of patients with HIV infection (25–27). In this study, we show that C. albicans infection is a major determinant of Vδ1 T cell expansions in patients with HIV, and we confirm that C. albicans drives expansion of and IL-17 production by human Vδ1 T cells in vitro. Although some Vδ1 T cells could produce IL-17 upon direct contact with the fungus, a robust IL-17 response to C. albicans, resulting in substantial IL-17 release, required DC-driven proliferation of Vδ1 T cells. C. albicans potently induced IL-1β, IL-6, and IL-23 secretion by DCs, but without the presence of DCs, these cytokines were not sufficient to induce Vδ1 T cell proliferation, resulting in elevated...
IL-17 secretion, which required direct contact with DCs as well as IL-23 signaling. These results indicate that V61 T cells are likely to be important mediators of immunity against candidiasis, particularly in the setting of HIV infection where CD4+ Th17 cells are depleted.

Materials and Methods

Subjects

Blood samples were obtained from 23 HIV-1–infected subjects with no evidence of Candida coinfection (16 white, 6 African, and 1 Asian subject; 13 male subjects) and 14 HIV-1–infected subjects with candidiasis (9 white and 5 African subjects; 8 male subjects), who were attending the Genito-Urinary and Infectious Diseases Clinic at St. James’s Hospital, Dublin, Ireland. Ten HIV+ healthy donors were also studied. All HIV-infected patients were receiving antiretroviral treatment. The CD4 T cell count at the time of blood collection ranged from 55 to 1115 (median 529) per microliter of blood in the patients without candidiasis and 261–1857 (median 575) per microliter in the patients with candidiasis. Eleven patients without candidiasis and eight with candidiasis had HIV viral loads of <50 copies/ml, and the remainder had viral loads ranging up to 72,976 copies/ml in the patients without candidiasis and 32,265 copies/ml in the patients with candidiasis. Three patients without candidiasis were positive for hepatitis B virus surface Ag and two were positive for hepatitis C virus RNA, whereas none of the patients with candidiasis had hepatitis B and one had hepatitis C. Ethical approval for this study was obtained from the Joint Research Ethics Committee of St. James’s Hospital and Tallaght Hospitals, Dublin, and all participants gave written, informed consent. Buffy coat packs from healthy blood donors were kindly provided by the Irish Blood Transfusion Service. PBMCs were prepared by density gradient centrifugation over Lymphoprep (Nycomed Pharma, Oslo, Norway) and used immediately in all procedures.

Abs and flow cytometry

Fluorochrome-conjugated mAbs specific for human V61 TCR (clone TS-1), CD3e, CD11c, CD14, IFN-γ, IL-17A, and dectin-1 were obtained from Thermo Fisher Scientific (Dublin, Ireland) and Biologend (San Diego, CA). A total of 10^6 cells were labeled with mAbs and analyzed using a CyAN ADP (Beckman Coulter, High Wycombe, U.K.) or FACSCanto (Becton Dickinson, Oxford, U.K.) flow cytometer. Data were analyzed with FlowJo v7.6 (Tree Star, Ashland, OR) software. Single-stained OneComp Beads (Becton Dickinson) were used to set compensation parameters, and fluorescence minus one and isotype-matched Ab controls were used to set analysis gates.

Preparation of fungi

C. albicans strain 10231 was obtained from the American Type Culture Collection and cultured on malt extract agar according to American Type Culture Collection protocols. Fungi were cultured for 24 h, isolated, counted, and then inactivated by heating at 96°C for 60 min or treating with 70% ethanol for 30 min. Samples were then centrifuged at 5000 × g for 10 min, the supernatants discarded, and the pellets washed with PBS. Inactivation was confirmed by plating an aliquot onto malt extract agar and incubating for 1–7 d to check for growth.

Generation of DCs

Monocytes were enriched from PBMCs isolated from buffy coat packs and allowed to differentiate into immature DCs by culturing them for 6 d in the presence of GM-CSF and IL-4 as described previously (28). Maturation of DCs was achieved by plating immature DCs at densities of 100,000/ml and stimulating them overnight with medium only, with the dectin-1 ligand curdlan (100 μg/ml; InvivoGen, Toulouse, France), or with heat- or ethanol-killed C. albicans (5 × 10^6 cells/ml).

In vitro expansion of V61 T cells

Total γδ T cells were enriched from healthy donor PBMCs using human anti-TCR γδ Microbeads (Miltenyi Biotec, Gladbach Bergische, Germany). γδ-enriched cells (200,000/ml) were cultured in the absence or presence of C. albicans– or curdlan-treated DCs at 2:1 ratios in complete serum-free AIM-V medium (AIM-V containing 0.05 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.02 M HEPES, 55 μM 2-ME, 1X essential amino acids, 1X nonessential amino acids, and 1 mM sodium pyruvate). Cocultures were challenged with PHA-L (1 μg/ml; Sigma-Aldrich, Dublin, Ireland) and cultured with rIL-2 (40 U/ml; Miltenyi Biotec), which was added in fresh medium every 2–3 d. Cultures were restimulated every 2 wk with activated DCs and PHA-L, which resulted in yields of >10 million V61 T cells by day 28.

V61 T cell stimulation and analysis of cytokine production

IFN-γ and IL-17 expression by fresh, unexpanded V61 T cells within γδ T cell–enriched PBMCs was examined by flow cytometry (29) after stimulation of the cells for 6 h with medium alone, 1 ng/ml PMA with 1 μg/ml ionomycin, or C. albicans in the absence or presence of DCs. Cytokine expression by V61 T cells that were expanded for 14 d with C. albicans–treated DCs was similarly examined. Levels of soluble IFN-γ, IL-1β, IL-6, IL-17A, IL-12, and IL-23 released into supernatants of DCs treated for 24 h with medium, C. albicans, or curdlan were measured by ELISAs using Ab pairs purchased from BioLegend (San Diego, CA) and R&D Systems (Abingdon, U.K.). ELISA was also used to measure cytokines released by V61 T cell–enriched PBMCs after treatment for up to 14 d with curdlan– or C. albicans–treated DCs, supernatants of immature or activated DCs, or rIL-1β (10 ng/ml; Immunotools, Friesoythe, Germany) or rIL-23 (10 ng/ml; eBioscience, Hatfield, U.K.). In some experiments, a blocking Ab specific for human IL-23R or isotype control Ab (20 μg/ml; eBioscience) was added at times 0, 3, 6, 17, and 3 d.

Statistical analysis

 Prism GraphPad software (San Diego, CA) was used for data analysis. V61 T cell frequencies in subject groups and cytokine levels in treatment groups were compared using the Mann–Whitney U test. Matched donor treatments were compared using the Wilcoxon matched pair test. The p values <0.05 were considered significant.

Results

V61 T cells are expanded in the circulation of HIV-infected subjects with candidiasis

Previous studies have demonstrated that V61 T cells are expanded in the circulation of patients with HIV infection (25–27). We used flow cytometry to determine the percentages of T cells that express V61 TCRs in fresh PBMCs from 10 healthy donors, 23 treated HIV-infected subjects without Candida coinfection, and 14 HIV-infected subjects with candidiasis. Fig. 1 confirms that V61 T cells are expanded in HIV-infected subjects compared with healthy donors and further shows that these cells are significantly more frequent in HIV-infected subjects with candidiasis compared with patients with no evidence of fungal infection (means 4.0

FIGURE 1. V61 T cells are expanded in the circulation of HIV-infected subjects with candidiasis. Frequencies of V61 T cells, as percentages of all T cells, in blood samples taken from 10 healthy donors, 23 HIV-infected subjects with no evidence of Candida coinfection, and 14 HIV-infected subjects with candidiasis. Freshly isolated PBMCs were stained with mAbs specific for CD3 and the V61 TCR chain, and the percentages of CD3+ cells that expressed V61 were determined by flow cytometry. Horizontal lines show the mean and error bars show SEM.
versus 1.0%; \( p < 0.001 \)). V61 T cell frequencies were only moderately increased in patients with HIV without candidiasis compared with healthy control subjects (0.32%; Fig. 1; NS). Therefore, \( C. \) albicans infection is likely to be a major driver of V61 T cell expansion in patients with HIV.

**FIGURE 2.** \( C. \) albicans drives the expansion of V61 T cells in vitro. (A) Representative flow-cytometry dot plots showing CD3ε and V61 TCR expression by freshly isolated PBMCs (top left panel), PBMCs enriched for total \( \gamma \delta \) T cells by magnetic bead separation (top center panel), \( \gamma \delta \) T cell–enriched PBMCs after stimulation for 7 d with monocyte-derived DCs pulsed overnight with vehicle (top right panel), 100 \( \mu \)g/ml curdlan (bottom left panel), or 5 \( \times \) \( 10^6 \) bodies/ml heat-inactivated (bottom center panel) or ethanol-inactivated (bottom right panel) \( C. \) albicans in the presence of 1 \( \mu \)g/ml PHA and 40 U/ml IL-2. Numbers indicate the percentages of total cells that express CD3+V61+ phenotypes. (B) Expansion of V61 T cells from \( \gamma \delta \) T cell–enriched PBMCs in the presence of PHA and IL-2 and in the presence and absence of DCs and \( C. \) albicans. Results show mean \( \pm \) SEM of four independent experiments. (C) Kinetics of V61 T cell expansion showing purities (left panel) and absolute numbers (right panel) of V61 T cells obtained after stimulation as described earlier on days 0 and 14, and analysis by flow cytometry on days 0, 7, 14, 21, and 28. Results show mean \( \pm \) SEM of four independent experiments.
scribed earlier on day 14, which resulted in yields of up to 10^7 V61 T cells by day 28 starting with 200,000 γδ T cell–enriched PBMCs (Fig. 2C). Purified populations of V61 T cells could be obtained by sorting CD3^+ γδ^+ T cells on a MoFlo XDP Cell Sorter (Beckman Coulter). This method proved to be a robust method for generating human V61 T cells lines.

C. albicans induces IL-1β, IL-6, and IL-23 secretion by DCs and downstream IFN-γ and IL-17 secretion by expanded V61-enriched T cells

To identify the cytokines produced in response to C. albicans stimulation of DCs and the downstream activation of enriched V61 T cells, we pulsed monocyte-derived DCs for 24 h in medium alone or with heat-inactivated C. albicans. Cell supernatants were collected after 7 and 14 d and analyzed for cytokine levels by ELISA. Fig. 3 shows that addition of C. albicans to immature DCs resulted in potent secretion of IL-1β, IL-6, IL-22, and IL-23, but not IFN-γ or IL-17A within 24 h. Addition of γδ T cell–enriched PBMCs resulted in secretion of IFN-γ and IL-17A, the levels of which peaked by day 7. The levels of IL-1β, IL-6, and IL-23 decreased over the 14-d period of culture with the V61-enriched T cells, presumably because they were mainly produced by C. albicans–treated DCs and not by T cells, and then were partially removed when medium was collected for analysis by ELISA and replaced with fresh medium. IL-1β, IL-6, and IL-23 levels may also have decreased because of consumption by the T cells. In contrast, IL-22 levels remained relatively constant over this 14-d period, suggesting that IL-22 is being produced both by DCs and T cells (Fig. 3). These data indicate that C. albicans directly induces proinflammatory cytokine production by DCs and that C. albicans–treated DCs induce IFN-γ and IL-17 secretion by γδ T cell–enriched PBMCs.

C. albicans induces IL-17 secretion by V61 T cells

The expanded γδ T cells used earlier typically contained <60% V61 T cells; therefore, it is possible that cells other than V61 T cells were releasing the IFN-γ and IL-17. To ascertain whether V61 T cells can produce IL-17 in response to C. albicans, we stimulated freshly isolated PBMCs enriched for γδ T cells for 6–12 h with C. albicans in the absence or presence of DCs, in the presence of monensin. Intracellular expression of IL-17A by V61 T cells was then analyzed by flow cytometry (Fig. 4A, 4B). Up to 10% of unexpanded V61 T cells expressed intracellular IL-17A when stimulated with C. albicans, alone or loaded onto DCs, but no IL-17A was detected in V61 T cells cultured with PMA and ionomycin or with DCs in the absence of C. albicans. When expanded V61 T cells, generated by treatment of γδ T cell–enriched

FIGURE 4. C. albicans induces IL-17 secretion by V61 T cells. γδ T cells were enriched from PBMCs by positive selection using magnetic beads. (A and B) Fresh γδ T cell–enriched PBMCs were treated for 6 h with medium alone, PMA and ionomycin, C. albicans, DC, or C. albicans–treated DCs, and intracellular expression of IL-17A by V61 T cells was then analyzed by flow cytometry. (A) Flow-cytometry dot plots showing IL-17 expression by V61 T cells treated with (from left to right) medium, PMA and ionomycin, C. albicans, DC, or C. albicans–treated DCs after gating on CD3^+ cells. Numbers in the plots show percentages of V61 T cells and non-V61 T cells that produced IL-17. (B) Mean (± SEM) frequencies of fresh V61 T cells from four γδ T cell–enriched PBMC samples that expressed IL-17A after the different treatments. (C-G) V61 T cells were expanded from γδ T cell–enriched PBMCs by treatment for 14 d with C. albicans–treated DCs in the presence of PHA and IL-2. Cells were then restimulated with PMA and ionomycin, and IL-17, IL-22, CD161, ROryt, and dectin-1 expression by V61 T cells were examined by flow cytometry. (C) Flow-cytometry dot plot showing IL-17 expression by V61 T cells expanded with C. albicans–treated DCs. (D) Mean (± SEM) frequencies of expanded V61 T cells in four expanded V61 T cell lines that expressed IL-17 after treatment with DCs alone, C. albicans–treated DCs, and curdlan-treated DCs. (E) Flow-cytometric analysis of CD161, IL-22, and ROryt expression by C. albicans–expanded V61 T cells. (F) Flow-cytometric detection of dectin-1 expression by C. albicans–expanded V61 T cells (right) after gating on CD3^+ cells. The left panel shows a fluorescence minus one (FMO) control omitting the dectin-1 mAb. (G) Mean ± SEM frequencies of IL-17 and IL-17^+ V61 T cells, expanded with C. albicans– or curdlan-pulsed DCs that expressed dectin-1. Results show mean ± SEM of results from experiments with three V61 T cell lines.
stimulation with C. albicans addition of DCs. However, Fig. 4C–E clearly shows that a 14-d related orphan receptor gamma t (RORγt)/markers of Th17 cells, such as CD161, IL-22, and retinoic acid-suppression by Vγd T cells in the absence of Vγd T cells although it cannot be ruled out that residual DCs or monocytes remaining in the γδ T cell–enriched PBMCs provided the necessary signals, this is unlikely because C. albicans stimulated IL-17 expression by Vγd T cells within 6 h and this was not augmented by the addition of DCs. However, Fig. 4C–E clearly shows that a 14-d stimulation with C. albicans–treated DCs is required to generate large numbers of IL-17A–producing Vγd T cells that coexpress markers of Th17 cells, such as CD161, IL-22, and retinoic acid-related orphan receptor gamma t (RORγt). Vγd T cells that produce IL-17A were also expanded (Fig. 4C), and these were found to express CD161 but did not include other γδ T cells (data not shown). Analysis of dectin-1 expression by these Vγd T cells indicated that most IL-17–producing Vγd T cells expressed this receptor, whereas the IL-17γd T cells did not (Fig. 4E, 4G), indirectly suggesting that C. albicans can directly stimulate IL-17 production by Vγd T cells (Fig. 4), both DCs and C. albicans are required for the induction of potent and sustained IL-17 release by Vγd T cells (Fig. 3).

IL-17 response to C. albicans requires DCs

Because DCs promoted sustained IL-17 secretion by C. albicans–treated Vγd T cells (Fig. 3) but were not required for intracellular expression of IL-17 by C. albicans–stimulated fresh Vγd T cells (Fig. 4), we further investigated the role of DCs in this IL-17 response. γδ T cell–enriched PBMCs were cultured, in the presence of PHA and IL-2, with C. albicans alone, DCs that were pulsed overnight with heat-inactivated C. albicans, or with supernatants of untreated or C. albicans–treated DCs. Culture supernatants were collected after 7 d for measurement of IFN-γ and IL-17 release by ELISA. Although C. albicans–treated DCs induced IL-17 release by Vγd T cells, Fig. 5A shows that neither C. albicans alone nor supernatants of untreated or C. albicans–treated DCs induced IL-17 production. In contrast, detectable IFN-γ was released by γδ T cell–enriched T cells stimulated with supernatants of C. albicans–treated DCs (Fig. 5B). Thus, C. albicans alone or soluble factors released by C. albicans–treated DCs, such as IL-1β, IL-6, and IL-23, are not sufficient to induce sustained IL-17 release by Vγd T cells in the absence of Vγd T cells.

Proliferation of Vγd T cells in response to C. albicans requires direct contact with DCs

We next tested the hypothesis that the IL-17 response of Vγd T cells to C. albicans requires DCs to induce Vγd T cell prolif-
oration to generate sufficient numbers of cells releasing IL-17 to allow detection by ELISA. Enriched γδ T cells that contain ∼5000 V61 T cells were cultured for 7 d with PHA and IL-2, in the presence of either C. albicans–treated DCs, supernatants of untreated or C. albicans–treated DCs (added at days 0, 3, and 5 of culture), or supernatants of C. albicans–treated DCs in the presence of C. albicans. The numbers of viable V61 T cells in the cultures were determined by a combination of microscopic cell counting and analysis by flow cytometry. Fig. 6A shows that, like for IL-17 production, proliferation of V61 T cells required C. albicans–treated DC and could not be restored by substituting them with supernatants of untreated or C. albicans–treated DCs, even in the presence of C. albicans. We also found that recombinant IL-1β, IL-23, or IL-1β+IL-23, added at time 0 and day 4 of culture at concentrations similar to those released by C. albicans–treated DCs, failed to restore full proliferation of V61 T cells (Fig. 6B).

Furthermore, enriched γδ T cells cultured for 7 d with PHA and IL-2 in the presence of C. albicans– or curdlan-treated DCs, but separated using a transwell insert, also failed to proliferate (Fig. 6C). These experiments confirm that direct contact with DCs is required for C. albicans–induced proliferation of V61 T cells.

**Discussion**

V61 T cells are thought to play a role in immunity against HIV. They are expanded in the circulation of patients with HIV infection (25–27). They can kill HIV-infected and -uninfected CD4+ T cells (32, 33) and can secrete cytokines that suppress replication of HIV-1 in T cell lines in vitro (34). In this study, we show that C. albicans infection is a major determinant of V61 T cell expansion in patients with HIV, with V61 T cells being present in significantly higher numbers in patients with HIV and candidiasis compared with patients with HIV with no evidence of Candida infection. Indeed, V61 T cells were only marginally expanded in HIV-infected patients without candidiasis compared with healthy donors. We also confirm a previous report (17) that C. albicans drives expansion and IL-17 production by human V61 T cells in vitro. We initially tested several protocols (30, 31) for expanding V61 T cells and found that coculturing PBMCs enriched for γδ T cells with DCs activated with the dectin-1 ligand curdlan in the presence of PHA and IL-2 resulted in the selective expansion of V61 T cells. Subsequently, it was found that substituting curdlan with heat- or ethanol-inactivated C. albicans, also known to bind dectin-1 (18–20), could similarly promote V61 T cell expansion in vitro. DCs were required for V61 T cell expansion, and cells could be restimulated every 14 d to induce further expansion and to maintain V61 T cell–rich lines for >6 wk.

Host defense against C. albicans requires IL-17 (5, 6) and IL-17–producing cells, including CD4+ Th17 cells (7) and IL-17–secreting innate lymphoid cells (35). γδ T cells have been shown to be an important source of IL-17 in mice (8, 20–22) and humans (17), and IL-17–producing γδ T cells from both species expand in response to C. albicans (17, 20). Purified murine γδ T cells can release IL-17 in response to curdlan stimulation in vitro and this response is augmented by addition of IL-23 (20). They can also release IL-17 in response to stimulation with IL-1β and IL-23 or IL-18 and IL-23 in the absence of TCR or dectin-1 stimulation (21, 22). This study using human γδ T cell–enriched PBMCs shows that C. albicans induces IL-1β, IL-6, and IL-23 secretion by DCs and downstream IFN-γ and IL-17 secretion by V61 T cells. However, neither IL-1β nor IL-23 alone or together was able to induce expansion of V61 T cells or significant IL-17 secretion as detected by ELISA. Furthermore, supernatants of
C. albicans–stimulated DCs, which contain all cytokines produced by C. albicans–treated DCs, failed to induce IL-17 production by Vδ1 T cells. We found that a strong IL-17 response to C. albicans was seen only in the presence of DCs and after Vδ1 T cells had proliferated.

Significant and sustained IL-17 production by Vδ1 T cells in response to C. albicans required DC-driven proliferation of Vδ1 T cells. However, we found that a proportion of fresh, unexpanded Vδ1 T cells within PBMCs expressed intracellular IL-17 in response to stimulation with C. albicans. This occurred within 6 h of stimulation in the absence of DCs and it was not augmented by the presence of DCs, suggesting that C. albicans can directly induce IL-17 production by Vδ1 T cells. The β-glucan receptor dectin-1 was expressed by most IL-17–producing Vδ1 T cells expanded with C. albicans, adding further support to this notion; however, blocking studies are required to confirm a role for dectin-1 in Vδ1 T cell activation by C. albicans. DCs were required to induce proliferation of Vδ1 T cells to generate sufficient numbers of IL-17–secreting cells to detect IL-17 in the supernatants of 7-d culture. T cell activation by IL-1

T cells that expressed markers of Th17 cells, such as CD161 and IL-23 is also a key factor in IL-17 production by Vδ1 T cells in response to C. albicans. Blocking the IL-23R potently directs the differentiation program of proinflammatory IL-17+ T helper cells. Thus, C. albicans can directly induce IL-17 production by Vδ1 T cells, but DCs are required for Vδ1 T cell proliferation to generate high numbers of IL-17–producing cells.

IL-23 is required for the induction of IL-17 production by murine γδ T cells (21, 22), by C. albicans–stimulated innate lymphoid cells (35), and by human CD4+ T cells (7). We found that DC–derived IL-23 is also a key factor in IL-17 production by Vδ1 T cells in response to C. albicans. Blocking the IL-23R potently inhibited IL-17 release, but not proliferation, of Vδ1 T cells. Taken together, our data show that IL-23 is an important factor for IL-17 production by C. albicans–stimulated human Vδ1 T cells, and that contact with DCs plays a key role in expanding C. albicans–responsive Vδ1 T cells to numbers sufficient to release significant amounts of IL-17.

Therapeutic inhibition of the IL-23/IL-17 axis is currently under consideration for inflammatory and autoimmune diseases (36, 37). In contrast, enhancement of these pathways may be beneficial for the treatment of C. albicans infection. The IL-23/IL-17 axis is also thought to contribute to immunity against HIV; therefore, stimulation of these pathways may also be of particular benefit to patients coinfected with HIV and C. albicans (38–40). HIV infects and kills IL-17–secreting CD4+ T cells, but Vδ1 T cells are expanded in patients with HIV infection (25–27). Therefore, Vδ1 T cells, which are currently under investigation as effectors in tumor immunotherapy (41), have potential for restoring IL-17 responses in patients with HIV/AIDS.

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Disclosures

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